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TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

1422-386PCT

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/367052

INTERNATIONAL APPLICATION NO.

PCT/JP97/00299

INTERNATIONAL FILING DATE

07 February 1997

PRIORITY DATE CLAIMED

TITLE OF INVENTION

NOVEL MOUSE CXC CHEMOKINE RECEPTOR

APPLICANT(S) FOR DO/EO/US

KISHIMOTO, Tadimitsu; NAGASAWA, Takashi; TACHIBANA, Kazunobu; IIZASA, Hisashi; YOSHIDA, Nobuaki; NAKAJIMA, Toshihiro; YOSHIE, Osamu

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
- a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☒ has been transmitted by the International Bureau.
- c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(3)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)).
- a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☐ have been transmitted by the International Bureau.
- c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
- d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98./International Search Report
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
- ☒ A SECOND or SUBSEQUENT preliminary amendment. /Sequence Listing
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

1.) Fourteen (14) Sheets of Formal Drawings

09/367052

PCT//JP97/00299

1422-386PCT

- 17.
- ☒
- The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):

Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO. \$970.00

International preliminary examination fee (37 CFR 1.482) not paid to
USPTO but International Search Report prepared by the EPO or JPO \$840.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO
but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. \$760.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO
but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$670.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO
and all claims satisfied provisions of PCT Article 33(1)-(4) \$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(e)).

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total Claims	37 - 20 =	17	X \$18.00
Independent Claims	7 - 3 =	4	X \$78.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable) Yes			+ \$260.00

TOTAL OF ABOVE CALCULATIONS =

Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity statement
must also be filed (Note 37 CFR 1.9, 1.27, 1.28).

SUBTOTAL =

Processing fee of \$130.00 for furnishing the English translation later than ☐ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(f)).

TOTAL NATIONAL FEE =

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +

TOTAL FEES ENCLOSED =Amount to be:
refunded \$

charged \$

- a.
- ☒
- A check in the amount of \$
- 1718.00
- to cover the above fees is enclosed.

- b.
- ☐
- Please charge my Deposit Account. No. _____ in the amount of \$ _____ to cover the above fees.
-
- A duplicate copy of this sheet is enclosed.

- c.
- ☒
- The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
-
- overpayment to Deposit Account No.
- 02-2448
- .

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:

Birch, Stewart, Kolasch & Birch, LLP
P.O. Box 747
Falls Church, VA 22040-0747
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SIGNATURE

[Signature] #32868
MURPHY, GERALD M., JR.
NAME

#28,977

REGISTRATION NUMBER

/GMM/sas August 6, 1999

PATENT
1422-386PCT

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: KISHIMOTO, Tadadmitsu et al
Int'l. Appl. No.: PCT/JP97/00299
Appl. No.: New Group: Unknown
Filed: August 6, 1999 Examiner: Unknown
For: NOVEL MOUSE CXC CHEMOKINE RECEPTOR

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION

Assistant Commissioner for Patents
Washington, DC 20231

August 6, 1999

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/JP97/00299 which has an International filing date of February 7, 1997, which designated the United States of America.--

IN THE CLAIMS:

CLAIM 6: Lines 1 and 2, change "any one of claims 1 to 5" to
--claim 1--

CLAIM 10: Line 2, change "any one of claims 1 to 5" to
--claim 1--

CLAIM 14: Line 2, change "any one of claims 6 to 9" to
--claim 6--

CLAIM 16: Lines 1 and 2, change "any one of claims 6 to 9" to
--claim 6--

CLAIM 17: Line 5, change "any one of claims 6 to 9" to
--claim 6--

CLAIM 19: Line 5, change "any one of claims 6 to 9" to
--claim 6--

CLAIM 20: Line 6, change "any one of claims 6 to 9" to
--claim 6--

CLAIM 22: Line 3, change "any one of claims 6 to 9" to
--claim 6--

CLAIM 23: Line 4, change "any one of claims 6 to 9" to
--claim 6--

REMARKS

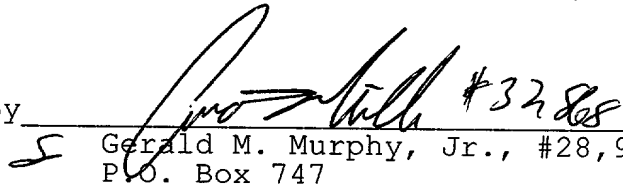
The specification has been amended to provide a cross-reference to the previously filed International Application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By


Gerald M. Murphy, Jr., #28,977
P.O. Box 747
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GMM/sas

(Rev. 03/30/99)

BOX SEQUENCE
PATENT
1422-386P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant(s): Tadimitsu KISHIMOTO et al.
Application No.: NEW Group No.: Not Assigned
International Application No. PCT/JP97/00299
Filed: August 6, 1999 Examiner: Not Assigned
For: NOVEL MOUSE CXC CHEMOKINE RECEPTOR

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

August 6, 1999

Dear Sir:

Preliminary to the examination of the above-referenced U.S. patent application, the following amendments and remarks are respectfully submitted.

IN THE SPECIFICATION:

Please amend the Specification as follows:

Page 4

Line 22, after "(Figure 1" insert --, (SEQ ID NO: 22)--

Page 10

Line 23, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Page 11

Line 6, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Page 12

Line 7, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

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Line 15, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Page 15

Line 18, after "cDNA" insert --(SEQ ID NO: 21)--

Line 18, after "amino acid sequence" insert --(SEQ ID NO: 22)--

Page 19

Line 20, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Page 20

Line 3, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Page 23

Line 10, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Line 18 change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Page 28

Line 12, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Line 20, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Page 41

Line 22, change "SEQ ID NO: 5" to --SEQ ID NO: 9--

Line 25, change "SEQ ID NO: 6" to --SEQ ID NO: 10--

Page 43

Line 6, change "SEQ ID NO: 2" to --SEQ ID NO: 3--

Line 9, change "SEQ ID NO: 7" to --SEQ ID NO: 11--

Line 10, change "SEQ ID NO: 8" to --SEQ ID NO: 12--

Line 15, change "SEQ ID NO: 3" to --SEQ ID NO: 5--

Line 23, change "SEQ ID NO: 15" to --SEQ ID NO: 19--

Page 44

Line 1, change "SEQ ID NO: 16" to --SEQ ID NO: 20--

Page 47

Line 7, change "SEQ ID NO: 4" to --SEQ ID NO: 7--

Line 10, change "SEQ ID NO: 3" to --SEQ ID NO: 5--

Line 11, change "SEQ ID NO: 4" to --SEQ ID NO: 7--

Line 23, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Page 48

Line 23, change "SEQ ID NO: 11" to --SEQ ID NO: 15--

Line 24, change "SEQ ID NO: 12" to --SEQ ID NO: 16--

Page 49

Line 1, change "SEQ ID NO: 9" to --SEQ ID NO: 13--

Line 2, change "SEQ ID NO: 10" to --SEQ ID NO: 14--

Line 22, change "SEQ ID NO: 3" to --SEQ ID NO: 5--

Page 50

Line 1, change "SEQ ID NO: 13" to --SEQ ID NO: 17--

Line 2, change "SEQ ID NO: 14" to --SEQ ID NO: 18--

Please delete pages 66 through 79 of the specification containing the Sequence Listing. Please renumber the remaining pages of the specification, beginning with the claims, consecutively from page 66 of the specification. Please insert the Substitute Sequence Listing enclosed herewith immediately after the abstract.

IN THE CLAIMS:

Please amend the claims as follows:

Claim 1

Line 3, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Claim 2

Line 4, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Claim 7

Line 2, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Claim 8

Line 4, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

IN THE ABSTRACT:

Please amend the abstract as follows:

Line 5, change "SEQ ID NO: 17" to --SEQ ID NO: 2--

Remarks

Enclosed herewith in full compliance with 37 C.F.R. 1.821-1.825 is a Sequence Listing to be inserted into the specification as indicated above. The Sequence Listing in no way introduces new matter into the specification. Additional SEQ ID NOS: 21 and 22 can be found in Figure 1.

Also submitted herewith in full compliance with 37 C.F.R. 1.821-1.825 is a disk copy of the Sequence Listing. The disk copy of the Sequence Listing, file "1422-386.app", is identical to the paper copy, except that it lacks formatting.


Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any

additional fees requires under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By  #32,868
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JRR
GMM/JRR
1422-386P

Enclosures: Paper and Disk copy of Sequence Listing

- 1 -

DESCRIPTION

NOVEL MOUSE CXC CHEMOKINE RECEPTORTECHNICAL FIELD

5 The present invention relates to a novel murine CXC
chemokine receptor, and a murine chemokine receptor gene.
More particularly, it relates to a polypeptide encoded by
the gene, an expression vector carrying the gene, a
transformant into which the expression vector is
10 introduced, and a monoclonal antibody against the
polypeptide. Further, it relates to a method for
producing the polypeptide using the transformant.
Furthermore, it relates to a method of screening an
agonist or antagonist of chemokines, and a method of
15 screening an AIDS onset inhibitor or an HIV-1 infection
inhibitor.

BACKGROUND ART

20 When a tissue impairment takes place owing to
causation such as a bacterial or viral infection, a
physical or chemical trauma, an autoimmune disease, an
allergic disease or the like, an inflammatory reaction
accompanied with signs such as flare, edema, fever and
pain is induced, and accumulation and infiltration of
25 peripheral leukocytes are observed at the local

inflammation. The kinds of the leukocytes infiltrating on the site of an inflammation vary depending on the diseases. An acute inflammation such as an ordinary bacterial infection, an immunological complex deposition and a trauma involves accumulation and infiltration mainly of a neutrophile; a tubercular infection, a typhoid infection and a delayed hypersensitivity involve those mainly of a monocyte; and a viral infection involves those mainly of a lymphocyte, while an eosinophile and a basophile infiltrate accompanied with an immediate allergy and a parasite infection [Baggioloni, M. et al., *Immunol. Today*, 15, 127-133 (1994)]. Recently, there have been found that chemotactic factors of polypeptides having certain degrees of selectivity to leukocytes having chemotactic activities, the polypeptides having characteristic four cysteine residues. Since they are in a family, members of which are homologous in their amino acid sequences and related to each other also in terms of the biological activities, they are referred to as chemokines (having chemoattractant and cytokine activity) [Lindley, I. J. D. et al., *Immunol. Today*, 14-24 (1993)].

Four cysteine residues of a chemokine are linked with disulfide bonds between the first and third residues and between the second and fourth residues, respectively.

Since their characteristics are found on the biological

activities in which whether or not one additional amino acid is contained between the first and second cysteine residues, their subfamilies are distinguished by referring to as CXC chemokines and CC chemokines [Baggioloni, M. et al., *Adv. Immunol.*, **55**, 97-179 (1994)].

The CXC chemokines which have been found so far are PBSF/SDF-1; IL-8 [Yoshimura, T. et al., *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 9233-9237 (1987)]; NAP-2 [Walz, A. et al., *Biochem. Biophys. Res. Commun.*, **159**, 969-975 (1989)]; NAP-4; GRO α [Richmondo, A. et al., *J. Cell. Biochem.*, **36**, 185-198 (1988)]; GRO β [Haskill, S. et al., *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 77732-7736 (1990)]; GRO γ [Haskill, S. et al., (1990) *ibid*]; GCP-2 [Proost, P. et al., *J. Immunol.*, **150**, 1000-1010 (1993)]; ENA-78 [Wayz, A. et al., *J. Exp. Med.*, **174**, 1355-1362 (1991)]; PF-4 [Deuel, T. F. et al., *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 2256-2258 (1977)]; a human CXCR4/fusin/HUMSTSR [Feng, Y. et al., *Science*, **272**, 872-877 (1996)]; and IP-10 [Dewald, B. et al., *Immunol. Lett.*, **32**, 81-84 (1992)].

And the CC chemokines are MCP-1 [Yoshimura, T. et al., *J. Immunol.*, **142**, 1956-1962 (1989)]; MCP-2 [Chang, H. C. et al., *Int. Immunol.*, **1**, 388-397 (1989)]; MCP-3 [Van Damme, J. et al., *J. Exp. Med.*, **176**, 59-65 (1992)]; MIP-1 α [Obaku, K. et al., *J. Biochem.*, **99**, 885-894

(1986)]; MIP-1 β [Lipes, M. A. et al., *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9704-9708 (1988)]; RANTES [Schall, T. et al., *J. Immunol.*, **141**, 1018-1025 (1988)]; I-309 [Miller, M. D. et al., *J. Immunol.*, **143**, 2907-2916 (1989)]; and
5 eotaxin [Jose, P. et al., *J. Exp. Med.*, **179**, 881-887 (1994)].

Most of the CXC chemokines have chemotactic activities on a neutrophil but not on a monocyte. Most of the CC chemokines have chemotactic activities on a
10 monocyte but not on a neutrophil. In addition, as to other leukocytes such as an eosinophil, a basophil and a lymphocyte, there have been reported to have the chemotactic activities for some of CXC and CC chemokines. While CC chemokines including RANTES, MIP-1 α and MCP-1,
15 and IL-8, which is a CXC chemokine, have been found to possess chemotactic activities on human lymphocytes, none of them are chemotactic factors specific to lymphocytes.

It has been reported that murine PBSF/SDF-1 is a CXC chemokine which is identified as a murine pre-B-cell
20 growth-stimulating factor originally secreted from a murine bone marrow stromal cell line PA6 with its amino acid sequence (Figure 1) [Nagasawa, T. et al., *Proc. Natl. Acad. Sci. USA*, **91**, 2305-2309 (1994)]. In addition, recently, it has been clarified to have a potent
25 chemotactic activity also on a human T lymphocyte [Bleul, C. et al., *J. Exp. Med.*, **184**, 1101-1110].

Various studies have been conducted on receptors for chemokines. There have been reported IL-8RA, which is a receptor specific to IL-8; IL-8RB, which is a receptor for IL-8 and other CXC chemokines; CC CKR1, which is a
5 receptor specific to MIP-1 α and RANTES; CC CKR2A, which is a receptor specific to MCP-1; CC CKR2B, which is a receptor specific to MCP-1 and MCP-3; CC CKR3, which is a receptor specific to eotaxin, MCP-3, and RANTES
[Combadiere, C. et al., *J. Biol.*, 270, 16491-16494
10 (1995)]; and CC CKR5, which is a receptor specific to MIP-1 α , MIP-1 β and RANTES. Recently, CXCR4/fusin/HUMSTSR has been identified as a receptor for SDF-1 which is a human CXC chemokine.

In addition, among the chemokine receptors mentioned
15 above, there has been clarified that CC CKR5, CC CKR2B, CC CKR3 and CXCR4/fusin/HUMSTSR have functions as receptors for HIV-1 by acting cooperatively with CD4, a protein present on a cell membrane, and that an infection with HIV-1 mediated by each receptor is inhibited by the
20 ligands of these receptors.

Two characteristically different HIV-1s are involved in the infection with an HIV-1, which is an AIDS-causing virus, and in the onset of AIDS. A monocyte-tropic HIV-1 with which monocytes, macrophages and T lymphocytes are
25 mainly infected is involved in the viral proliferation in

5 a human body during the period of infection and latent
infection, and a T-cell-line-tropic HIV-1 with which T
lymphocytes are mainly infected is involved in the
reduction of the number of T lymphocytes and the onset of
AIDS. In order to infect cells with the two HIV-1s
mentioned above, two receptors are required. One is CD4
protein, which is a cell membrane protein, and is a common
receptor for the two HIV-1s mentioned above. The other is
a protein referred to as a coreceptor which has an
10 activity as a receptor by acting cooperatively with the
CD4 protein, and is specific to each of the two HIV-1s.

15 Recently, there has been clarified that a coreceptor
for a main monocyte-tropic HIV-1 is found to be CC CKR5,
which is a CC chemokine receptor, and a coreceptor for
T-cell-line-tropic HIV-1 is found to be human
CXCR4/fusin/HUMSTSR, which is a CXC chemokine receptor.
Further, there has been clarified that the infection with
a monocyte-tropic HIV-1 is inhibited by MIP-1 α , MIP-1 β and
RANTES, which are CC CKR5 ligands, and the infection with
20 a T-cell-tropic HIV-1 is inhibited by a human PBSF/SDF-1,
which is a human CXCR4/fusin/HUMSTSR ligand, suggesting
that the chemokine receptors described above could be a
target of an HIV-1 infection inhibitor.

25 On the other hand, which domain in a human
CXCR4/fusin/HUMSTSR is essential for the infection with a

5 T-cell-line-tropic HIV-1 has not been identified so far.
A CXC chemokine receptor, human CXCR4/fusin/HUMSTSR is a
seven transmembrane-spanning-domain receptor, and a
three-dimensional structure formed by four extracellular
domains is considered to be significant in the binding
with a ligand or an HIV-1. For the purpose of identifying
a functional domain of a human CXCR4/fusin/HUMSTSR, it is
necessary to produce a CXCR4/fusin/HUMSTSR variant so as
to maintain the three-dimensional structure as a receptor.
10 The identification of a functional domain of a human
CXCR4/fusin/HUMSTSR is extremely useful in the development
of an HIV-1 infection inhibitor.

15 In addition, the elucidation of the mechanism for
causation of an HIV-1 species-specificity is significant
in the development of an HIV-1 infection model animal, as
in the case of clarifying an intracellular factor required
for a viral infection. Although a mouse is an excellent
experimental animal which can easily be handled and
obtained at a low cost of which characteristics are
clarified in detail, there has been no report that a mouse
20 is infected with an HIV-1.

Murine cells have several barriers concerning an
HIV-1 viral infection. A first barrier is present on the
stage where a virus is bound to murine cells. A human CD4
25 is bound to an HIV-1, but a murine CD4 is not bound to an

HIV-1. However, there has been clarified in the existing studies that when a human CD4 is expressed in vitro on the surface of murine cell lines including a T-cell line, the adsorption of an HIV to the cells takes place, but an entry into the cells does not. It was clarified from the above results that the murine cells expressing a human CD4 do not support an entry of the virus, suggesting that there is a receptor, in addition to CD4, which is human-specific and essential for a membrane fusion (which takes place at an entry of the virus) of which molecule is absent in murine cells.

The HIV-1 is recognized to have a difference in an infection ability to CD 4-positive cells depending on the strains. Some strains are classified as monocyte- or macrophage-tropic (M-tropic) strains since they infect a monocyte, and others are classified as T-cell-line-tropic (T-tropic) strains since they infect a T-cell line.

As an HIV-1 infection is progressed, a monocyte-tropic virus observed frequently at an initial stage of the infection is replaced with a T-cell-line-tropic virus. In 1996, there has been reported that CXCR4/fusin, which is a seven transmembrane-spanning-domain G protein-coupled receptor, is essential for the entry of a T-cell-line-tropic HIV-1 into human CD4-positive cells. These results urged the

present inventors to study whether or not the function of the CXCR4 as a viral entry receptor is species-specific.

DISCLOSURE OF THE INVENTION

5 The present inventors have clarified that a murine CXCR4 as a receptor of a murine PBSF/SDF-1, which is one of CXC chemokines, is isolated, and found its amino acid sequence to be in 90% identity with a human CXCR4. The present inventors have established cells transfected with
10 a human CD4 and a murine CXCR4, and studied whether or not a human CXCR4, which is an HIV-1 receptor, serves as a barrier against an HIV-1 entry present in the murine cells.

 Accordingly, an object of the present invention is to
15 provide a novel murine CXC chemokine receptor gene; a polypeptide encoded by the gene; an expression vector carrying the gene; a transformant harboring the expression vector; a monoclonal antibody against the polypeptide; a method for producing the polypeptide; and a method of
20 screening an agonist or antagonist of the polypeptide and also a method of screening an HIV-1 infection inhibitor, each of which is useful in studies of a therapeutic agent for AIDS and the functional mechanism of HIV-1 infection.

 Specifically, as a result of intensive studies in
25 order to solve the problems described above, the present

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inventors have succeeded in cloning a novel murine chemokine receptor gene from a murine pre-B-cell line DW34 of which growth is enhanced depending on a murine PBSF/SDF-1. The present inventors have also found that
5 cells expressing murine CXCR4 and human CD4 fuse with cells expressing an env protein derived from a T-cell-line-tropic HIV-1 strain, and those cells are infected with such a T-cell-line-tropic HIV-1 strain. These results lead to a conclusion that CXCR4 is not a
10 species-specific barrier against the entry of a T-cell-line-tropic HIV-1, which is present in the murine cells. In addition, because of the fact that a T-cell-line-tropic HIV-1 chimera virus clone of which env or V3 region is replaced with that of a monocyte-tropic
15 HIV-1 does not infect cells expressing murine CXCR4 and human CD4 cells, there has been revealed that a V3 loop of an HIV-1 envelope protein is essential for a murine CXCR4-mediated HIV-1 entry. Based on these findings, the present invention has been completed.

20 In sum, the present invention pertains to:
[1] a DNA encoding a polypeptide comprising an entire sequence of the amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, or a polypeptide comprising the polypeptide
25 described above, wherein any of the polypeptides has an

activity of a receptor capable of binding to a murine
PBSF/SDF-1;

[2] a DNA encoding a polypeptide resulting from at least
one of deletion, addition, insertion, or substitution of
one or more amino acid residues in an entire sequence of
the amino acid sequence as shown by SEQ ID NO: 17 of
Sequence Listing or a partial sequence thereof, wherein
any of the polypeptides has an activity of a receptor
capable of binding to a murine PBSF/SDF-1;

[3] a DNA comprising an entire sequence of the nucleotide
sequence as shown by SEQ ID NO: 1 of Sequence Listing or a
partial sequence thereof, or a DNA comprising the DNA
described above, wherein any of the DNAs encodes a
polypeptide having an activity of a receptor capable of
binding to a murine PBSF/SDF-1;

[4] a DNA resulting from at least one of deletion,
addition, insertion, or substitution of one or more bases
in a DNA comprising an entire sequence of the nucleotide
sequence as shown by SEQ ID NO: 1 of Sequence Listing or a
partial sequence thereof, or a DNA comprising the DNA,
wherein any of the DNAs encodes a polypeptide having an
activity of a receptor capable of binding to a murine
PBSF/SDF-1;

[5] a DNA being capable of hybridizing under stringent
conditions with the DNA of any one of items [1] to [4]

above, and encoding a polypeptide having an activity of a receptor capable of binding to a murine PBSF/SDF-1;

[6] a polypeptide encoded by the DNA of any one of items [1] to [5] above, wherein the polypeptide has an activity of a receptor capable of binding to a murine PBSF/SDF-1;

[7] a polypeptide comprising an entire amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, or a polypeptide comprising the polypeptide described above, wherein any of the polypeptides has an activity of a receptor capable of binding to a murine PBSF/SDF-1;

[8] a polypeptide resulting from at least one of deletion, addition, insertion, or substitution of one or more amino acid residues in an entire amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, wherein the polypeptide has an activity of a receptor capable of binding to a murine PBSF/SDF-1;

[9] the polypeptide according to any one of items [6] to [8] above, derived from a murine pre-B-cell line DW34;

[10] an expression vector carrying the DNA according to any one of items [1] to [5] above;

[11] a transformant obtained by introducing the expression vector according to item [10] above into a host;

[12] the transformant according to item [11] above,

wherein the host is a mammalian cell line;

[13] a method for producing a polypeptide having an activity of a receptor capable of binding to a murine PBSF/SDF-1, characterized in that the method comprises culturing the transformant according to item [11] or [12]

5 above under conditions capable of expressing the expression vector according to item [10] above;

[14] a monoclonal antibody against the polypeptide according to any one of items [6] to [9] above;

10 [15] a pharmaceutical composition for the use as an AIDS onset inhibitor or an HIV-1 infection inhibitor, comprising a murine PBSF/SDF-1;

[16] cells expressing the polypeptide according to any one of items [6] to [9] above and a human CD4 protein;

15 [17] a method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, characterized in that the method comprises the steps of:

(a) mixing the cells expressing the polypeptide according to any one of items [6] to [9] above, or cells according to item [16] above; a human

20 T-cell-line-tropic HIV-1; and a substance to be screened, and incubating the resulting mixture; and

(b) analyzing localization of an HIV-1 in the cells;

[18] the method according to item [17] above, wherein the step of analyzing localization of an HIV-1 is carried out

25 by using a monoclonal antibody against a human

T-cell-line-tropic HIV-1;

[19] a method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, characterized in that the method comprises the steps of:

- 5 (a) mixing the cells expressing the polypeptide according to any one of items [6] to [9] above, or cells according to item [16] above; cells expressing an HIV-1 envelope protein; and a substance to be screened, and incubating the resulting mixture; and
- 10 (b) determining a level of the fusion of the above cells with the cells expressing an HIV-1 envelope protein;
- [20] a method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, or a PBSF/SDF-1 agonist or antagonist, characterized in that the method comprises the
- 15 steps of:
- (a) mixing the cells expressing the polypeptide according to any one of items [6] to [9] above, or cells according to item [16] above; a murine or human PBSF/SDF-1; and a substance to be screened, and
- 20 incubating the resulting mixture; and
- (b) determining an intracellular calcium ion level and/or determining a binding activity of an expressed polypeptide with the murine or human PBSF/SDF-1;
- [21] the method according to item [20] above, wherein the
- 25 antagonist is a hematopoietic stem cell liberator;

[22] a kit for detecting an AIDS onset or an HIV-1 infection, comprising the cells expressing the polypeptide according to any one of items [6] to [9] above, or cells according to item [16] above; and

5 [23] a method for detecting an AIDS onset or an HIV-1 infection, characterized in that the method comprises;

(a) mixing the cells expressing the polypeptide according to any one of items [6] to [9] above, or cells according to item [16] above with sera, blood cells

10 or blood of a patient suspected to be infected with an HIV-1, and incubating the resulting mixture, and

(b) analyzing localization of an HIV-1 in the cells or determining a level of the fusion of the cells with HIV-1-infected cells.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a nucleotide sequence of a murine PBSF/SDF-1 cDNA, and an amino acid sequence of a murine PBSF/SDF-1 encoding the above nucleotide sequence.

20 Figure 2 shows electrophoretic results of Example 2 by means of Northern blotting method, wherein A shows the results on mRNA of murine tissues; and B shows the results on mRNA of murine fetus.

Figure 3 is graphs each showing results of Example 6,

25 wherein the abscissa indicates the passage of period of

time, and the ordinate indicates the ratio of fluorescence intensities ([fluorescence intensity at 340 nm]/[fluorescence intensity at 380 nm]). The cells used are CHO cells in which the chemokine receptor is not expressed.

Figure 4 is graphs each showing results of Example 6, wherein the abscissa indicates the passage of period of time, and the ordinate indicates the ratio of fluorescence intensities ([fluorescence intensity at 340 nm]/[fluorescence intensity at 380 nm]). The cells used are CHO cells in which the human chemokine receptor CC CKR2B is expressed.

Figure 5 is graphs each showing results of Example 6, wherein the abscissa indicates the passage of period of time, and the ordinate indicates the ratio of fluorescence intensities ([fluorescence intensity at 340 nm]/[fluorescence intensity at 380 nm]). The cells used are CHO cells in which the receptor (murine CXCR4) of murine chemokine (PBSF/SDF-1) is expressed.

Figure 6 is graphs each showing results of Example 6, wherein the abscissa indicates the passage of period of time, and the ordinate indicates the ratio of fluorescence intensities ([fluorescence intensity at 340 nm]/[fluorescence intensity at 380 nm]). The cells used are CHO cells in which the human chemokine receptor

CXCR4/fusin/HUMSTSR is expressed.

Figure 7 is graphs each showing results of Example 6, wherein the abscissa indicates the passage of period of time, and the ordinate indicates the ratio of fluorescence intensities ([fluorescence intensity at 340 nm]/
5 [fluorescence intensity at 380 nm]). The cells used are CHO cells in which a murine CXCR4 is expressed.

Figure 8 is a graph showing that a murine CXCR4 supports a membrane fusion via env protein derived from a
10 human T-cell-line-tropic HIV-1 strain. NIH3T3, which is target cells, is subjected to infection with a recombinant vaccinia virus in which human CD4, T7 polymerase, and an ω -subunit of β -gal are expressed. After infection, these cells are transfected with a murine CXCR4, a human CXCR4,
15 or a human CCR5. HeLaS3, which is effector cells, is subjected to infection with a recombinant vaccinia virus in which env protein derived from NL 432 or SF 162 and an α -subunit of β -gal are expressed. After the resulting infected cells are subjected to cell fusion, the resulting
20 fusion product is subjected to β -gal assay.

Figure 9 is a graph showing that a murine CXCR4 supports an infection with a human T-cell-line-tropic HIV-1 virus. SW480 cells (A) are transfected with a human CD4, and each of chemokine receptors (murine CXCR4, human
25 CXCR4, human CCR5, and human CCR2b). The resulting

transfected cells are subjected to infection with each of NL432 strain, IIIB strain and SF162 strain of HIV-1. A cell lysate of each of the resulting infected cells is then subjected to β -gal assay.

5 Figure 10 is a graph showing that a murine CXCR4 supports an infection with a human T-cell-line-tropic HIV-1 virus. HOS cells (B) are transfected with a human CD4, and each of chemokine receptors (murine CXCR4, human CXCR4, human CCR5, and human CCR2b). The resulting
10 transfected cells are subjected to infection with each of NL432 strain, IIIB strain and SF162 strain of HIV-1. A cell lysate of each of the resulting infected cells is then subjected to β -gal assay.

15 Figure 11 is a graph showing that a murine CXCR4 supports an infection with a human T-cell-line-tropic HIV-1 virus. U87MG cells (C) are transfected with a human CD4, and each of chemokine receptors (murine CXCR4, human CXCR4, human CCR5, and human CCR2b). The resulting
20 transfected cells are subjected to infection with each of NL432 strain, IIIB strain and SF162 strain of HIV-1. A cell lysate of each of the resulting infected cells is then subjected to β -gal assay.

25 Figure 12 is a schematic view showing the structure of chimeric provirus clone, wherein env or V3 loop of SF162 is incorporated into a provirus DNA of NL432, which is a human

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T-cell-line-tropic HIV-1 strain, wherein E denotes EcoRI; Ba denotes BamHI; St denotes StuI; and Nh denotes NheI.

Figure 13 is a diagram showing that a V3 loop of an envelope protein gp 120 is essential in an entry of HIV-1 via a murine CXCR4. The SW480 cells expressing the human CD4 and the receptors shown in the figure are subjected to infection with NL432 strain and SF162 strain of HIV-1, and NL432 env-162 and NL432 V3-162 , which are chimeric provirus clones.

BEST MODE FOR CARRYING OUT THE INVENTION

1. DNA of the Present Invention

The DNA of the present invention is not particularly limited as long as it is a DNA encoding a murine PBSF/SDF-1 receptor (murine CXCR4), which is a novel murine CXC chemokine receptor. Concretely, the following DNAs are exemplified:

1) a DNA encoding a polypeptide comprising an entire sequence of the amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, or a polypeptide comprising the polypeptide described above, wherein any of the polypeptides has an activity of a receptor capable of binding to a murine PBSF/SDF-1;

2) a DNA encoding a polypeptide resulting from at least

one of deletion, addition, insertion, or substitution of one or more amino acid residues in an entire sequence of the amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, wherein
5 any of the polypeptides has an activity of a receptor capable of binding to a murine PBSF/SDF-1;

3) a DNA comprising an entire sequence of the nucleotide sequence as shown by SEQ ID NO: 1 of Sequence Listing or a partial sequence thereof, or a DNA comprising the DNA,
10 wherein any of the DNAs encodes a polypeptide having an activity of a receptor capable of binding to a murine PBSF/SDF-1;

4) a DNA resulting from at least one of deletion, addition, insertion, or substitution of one or more bases
15 in a DNA comprising an entire sequence of the nucleotide sequence as shown by SEQ ID NO: 1 of Sequence Listing or a partial sequence thereof, or a DNA comprising the DNA, wherein any of the DNA encodes a polypeptide having an activity of a receptor capable of binding to a murine
20 PBSF/SDF-1; and

5) a DNA being capable of hybridizing under stringent conditions with the DNA of any one of items 1) to 4) above, and encoding a polypeptide having an activity of a receptor capable of binding to a murine PBSF/SDF-1.

25 In addition, in item 2), the phrase "deletion,

addition, insertion, or substitution of one or more amino acid residues" is not particularly limited, which, for instance, refers to deletion, addition, insertion, or substitution of one or several amino acid residues. Here, the term "several" refers, for instance, to a number of 10 or less. Further, in item 4), the extent of deletion, addition, insertion, or substitution of the bases of the DNA of the present invention is one or more bases, preferably one to several bases. Here, the term "several" refers, for instance, to a number of 10 or less. In addition, as long as the function or activity of the polypeptide to be expressed is of the same level, there may be included amino acid residues or bases which are chemically or biochemically modified, or non-naturally occurring or derivatized.

The DNA of the present invention can be isolated by amplifying a nucleotide sequence having homology with a known chemokine receptor by PCR, and screening a murine cDNA library using the amplified fragment as a probe.

An experimental method which can be employed in the present invention involves general procedures employed in molecular biology (DNA electrophoresis, a method of recovery of an electrophoretically separated DNA from a gel, ligation, host transformation, culture of recombinant host, plasmid DNA preparation, DNA cleavage with

restriction enzymes, DNA radiolabelling and the like)
which are well known to one of ordinary skill in the art,
including, for instance, those described in *Molecular*
Cloning 2nd Ed. [Maniatis et al., Cold Spring Harbor
5 Laboratory, New York (1989)].

The primer used in PCR includes those obtained on the
basis of an amino acid sequence conserved in a reported
human chemokine receptor, and, for example, added with an
appropriate restriction enzyme site on a 5'-side of a
10 condensed forward primer to a DNA sequence encoding an
amino acid sequence of a second transmembrane-spanning
domain; or added with an appropriate restriction enzyme
site on a 5'-side of a condensed reverse primer to a DNA
sequence encoding an amino acid sequence of a seventh
15 transmembrane-spanning domain. These primers can be
synthesized with a DNA synthesizer.

Also, the murine mRNA used in a cDNA cloning can be
purified from cells from, for instance, a murine
pre-B-cell line DW34 (provided by Prof. Nishikawa of Kyoto
20 Univ.), and the like with a commercially available mRNA
purification kit.

In addition, the murine genomic DNA cloning can be
performed by using, for instance, a DNA fragment derived
from a cDNA of a murine CXCR4, and the like.

25 The nucleotide sequence of a cDNA or the nucleotide

sequence of a genomic DNA thus obtained is subjected to
its nucleic acid homology search, referring to, for
example, GenBank/EMBL/DDBJ DNA sequence data base, and
whereby whether or not the cDNA obtained encodes a
5 chemokine receptor can be deduced. SEQ ID NO: 1 in
Sequence Listing shows the nucleotide sequence of the cDNA
obtained. Since the nucleotide sequence spanning from
120-position to 1196-position in SEQ ID NO: 1 of Sequence
Listing is the longest open reading frame, the amino acid
10 sequence (SEQ ID NO: 17 of Sequence Listing) deduced on
the basis of the nucleotide sequence of this open reading
frame is subjected to its homology search, using a program
such as DNASIS (HITACHI, LTD.) or BLAST [Altschul, F. et
al., *J. Mol. Biol.*, 215, 403-410], to a database such as
15 Genbank, EMBL or DDBJ, whereby the polypeptide encoded by
the DNA of the present invention can be further studied.

As a result, the polypeptide having the amino acid
sequence as shown by SEQ ID NO: 17 of Sequence Listing has
been deduced to be a trimer G protein-coupled receptor
20 covering a seven transmembrane-spanning domain,
characteristic to a chemokine receptor. In addition, as a
result of comparison with the amino acid sequences of
known CXC chemokine receptors, there has been revealed
that a human CXCR4/fusin/HUMSTSR is most closely resembles
25 it (90% identity).

In addition, since cells in which the DNA of the present invention is expressed have had receptor activity to a chemokine (murine PBSF/SDF-1) as well as an intracellular calcium level-increasing activity, the DNA of the present invention has been found to encode the novel murine chemokine receptor, and the protein encoded by this DNA is named a murine CXCR4.

The term "chemokine" refers to, among causative substances for which leukocytes show chemotactic activity to the local inflammation reaction as described above, a family of polypeptides having certain degrees of selectivity for migrating leukocytes and having four characteristic cysteine residues. These polypeptides are related with each other in their amino acid sequences and the biological activities. Four cysteine residues of a chemokine form disulfide bonds respectively between the first and third residues and between the second and fourth residues. The chemokine carrying another amino acid between the first and second cysteine residues is referred to as a "CXC chemokine," which is differentiated from the chemokine which has no additional amino acids referred to as "CC chemokine." Generally, there has been known that the CC chemokine has a chemotactic activity on a monocyte, but not on a neutrophile, and that the CXC chemokine has a chemotactic activity on a neutrophile, but not on a

monocyte.

The term "chemokine receptor" refers to a family of cell membrane proteins bound specifically to the chemokines described above. The chemokine receptors are related with each other in their amino acid sequences and structures. All of the chemokine receptors have a seven transmembrane-spanning-domain characteristic to a rhodopsin family and a binding domain with a trimer G protein. The chemokine receptors are classified into two subgroups on the basis of the specificities to ligands. Among the chemokines described above, one bound specifically to the CXC chemokine is referred to as a "CXC chemokine receptor," which is differentiated from a "CC chemokine receptor" which is bound specifically to the CC chemokine. Generally, a chemokine receptor has an intracellular calcium level-increasing activity when bound to the respective ligand. Recently, there have been clarified that some chemokine receptors have not only an activity as a chemokine receptor but also an activity as an HIV-1 receptor by acting cooperatively with a molecule called CD4 which is present on a cell membrane.

In the present specification, the receptor activity to a murine PBSF/SDF-1 can be determined, for instance, in a manner as described below.

A PBSF/SDF-1 peptide which is a murine CXCR4 ligand

is labeled with ^{125}I using, for example, BOLTON-HUNTER reagent, or is labeled with an enzyme such as an alkaline phosphatase. The labeled PBSF/SDF-1 peptide is added to a suspension of cells expressing a polypeptide having
5 receptor activity, and incubated at a given temperature. After washing, the amount of the PBSF/SDF-1 peptide bound to the cells can be determined by quantifying the label, whereby assaying the receptor activity. Examples of the cells used herein are a murine pre-B-cell line DW34; human
10 fetal kidney cell line 293 cells, or CHO cells derived from a Chinese hamster ovary cell line treated so as to express a murine CXCR4, and the like.

In addition, it is preferable that the polypeptide of the present invention has an activity of increasing the
15 level of the intracellular calcium ions when bound to the ligand. The above activity can, for instance, be determined as described below.

The cells expressing the polypeptide, the subject for measurement of the above activity, are washed with a
20 buffer, and the washed cells are suspended in an appropriate buffer [comprising, for instance, HBSS (20 mM Hepes, 125 mM NaCl, 5 mM KCl, 1 mM MgCl_2 , 0.5 mM glucose, and 0.1% BSA at pH 7.4)]. A fluorescence reagent which is
25 likely to be affected by the intracellular calcium ions is added to the suspension and incubated, so that the cells

can be labeled. The labeled cells are washed with a buffer, and subsequently suspended in an appropriate buffer, so that the activity can be determined from the changes in the fluorescence intensities when a chemokine, which is a ligand, is added.

For instance, when fura-PE3AM (Texas Fluorescence Laboratories) is used as a fluorescence reagent, the determination is taken under conditions such that excitation wavelengths are 340 nm and 380 nm, a fluorescence wavelength is 510 nm, and a response is 0.5 seconds. Thereafter, the ratio of [fluorescence intensity at an excitation wavelength of 340 nm] to [fluorescence intensity at an excitation wavelength of 380 nm] is calculated. When the level of the intracellular calcium ions is increased in the cells to be measured by the addition of a chemokine, an increase in the ratio of the fluorescence intensities can be found. In addition, by adding different kinds of chemokines, the receptor specificities to the ligands can be also confirmed.

In addition, the presence of the mRNA in the murine CXCR4 can be confirmed by employing a usual mRNA specific detection method. For instance, the mRNA can be detected by Northern blotting analysis or *in situ* hybridization method by using an antisense RNA or cDNA as a probe.

Alternatively, the mRNA can be also detected by converting an mRNA to a cDNA with a reverse transcriptase, and then performing PCR by an appropriate combination of primers.

5 2. Polypeptide of the Present Invention

 The polypeptide of the present invention includes, for instance, the following:

- 10 1) a polypeptide encoded by the DNA of the present invention, wherein the polypeptide has an activity of a receptor capable of binding to a murine PBSF/SDF;
- 15 2) a polypeptide comprising an entire amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, or a polypeptide comprising the polypeptide described above, wherein any of the polypeptides has an activity of a receptor capable of binding to a murine PBSF/SDF;
- 20 3) a polypeptide resulting from at least one of deletion, addition, insertion, or substitution of one or more amino acid residues in an entire amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, wherein the polypeptide has an activity of a receptor capable of binding to a murine PBSF/SDF; and
- 25 4) the polypeptide according to any one of items 1) to 3) above, derived from a murine pre-B-cell line DW34.

 In Embodiment 3), the extent of deletion, addition,

insertion, or substitution of the amino acid residues of the polypeptide of the present invention is one or more, and the number of mutation is not particularly limited as long as the polypeptide has an activity of a receptor capable of binding to a murine PBSF/SDF-1. For instance, the number of mutations may be from one to several. Here, the term "several" refers to a number of, for instance, 10 or less. In addition, as long as the function or activity of the polypeptide is of the same level, there may be encompassed amino acid residues which are chemically or biochemically modified, or non-naturally occurring or derivatized.

In addition, it is preferable that the polypeptide of the present invention is those derived from a murine pre-B-cell line DW34.

The presence of the polypeptide of the present invention can be confirmed by employing a usual detection method for a specific protein, including, for instance, usual immunoprecipitation method, Western blotting method, or analysis by FACS each using an antibody specific to a murine CXCR4.

3. Expression Vector and Transformant of the Present Invention

The expression vector of the present invention can be

obtained by, for example, incorporating the DNA of the present invention into a known vector such as pEFBOS, pCAGGStkNeo, or pMX.

In addition, the transformant of the present invention can be obtained by introducing the expression vector of the present invention into a desired host. The host is not particularly limited, and is preferably a mammalian cell line. Examples of the mammalian cell line are a murine pre-B-cell line, a human fetal kidney cell line, a cell line derived from a Chinese hamster ovary, and the like, and the cell line derived from a hamster ovary is preferable. A method for introducing an expression vector into a host may be any known method, including, for instance, such as a calcium phosphate method, a DEAE dextran method and an electroporation method.

In addition, the transformant described above is cultured under conditions capable of expressing the expression vector, whereby producing a polypeptide having an activity of a receptor capable of binding to the murine PBSF/SDF-1. The polypeptide produced in the manner described above can readily be purified by a usual column chromatography or an affinity chromatography using the antibody of the present invention.

4. Monoclonal Antibody of the Present Invention

Examples of the monoclonal antibody of the present invention are ones against the murine CXCR4 polypeptide and ones against a fusion protein of the above polypeptide with a human CXCR4/fusin/HUMSTSR.

The above monoclonal antibody can be prepared by a method described below.

As an immunogen, there are employed a synthetic polypeptide prepared by a usual peptide synthesizer based on a part of the amino acid sequence of the polypeptide of the present invention, or a murine CXCR4 produced by bacterial cells, yeasts, animal cells and insect cells which have been transformed with a vector expressing a murine CXCR4 in the form of cells themselves or a protein obtained by purifying with a usual protein chemistry technique. The above immunogen is used to immunize an animal such as a mouse, a rat, a hamster, a rabbit, or the like, and cells are collected from a spleen or a lymph node to be fused with myeloma cells, to prepare a hybridoma in accordance with a method described in Koehler and Milstein [*Nature*, 256, 495-497 (1975)] or a modification thereof described in Ueda et al. [*Proc. Natl. Acad. Sci. USA*, 79, 4386-4390 (1982)]. The hybridoma can produce a monoclonal antibody.

More concretely, for example, a monoclonal antibody

to a murine CXCR4 can be obtained by the following steps.

- (a) immunizing a mouse with a murine CXCR4 protein;
- (b) enucleating an immunized murine spleen and separating spleen cells;
- 5 (c) fusing separated spleen cells with murine myeloma cells in the presence of a fusion enhancing agent (for example, polyethylene glycol) in accordance with a method described in Koehler et al. above;
- (d) culturing hybridoma cells obtained in a selection
10 medium in which non-fusion myeloma cells do not grow;
- (e) selecting desired antibody-producing hybridoma cells by means of ELISA method, an immunoelectrotransfer method, and the like, and cloning the cells by a limiting dilution method; and
- 15 (f) collecting an anti-mouse murine CXCR4 monoclonal antibody.

In addition, the monoclonal antibody against a fusion protein of a murine CXCR4 with a human CXCR4/fusin/HUMSTSR
20 is also encompassed in the present invention.

The monoclonal antibody mentioned above can be obtained by producing a fusion protein of a murine CXCR4 with a human CXCR4/fusin/HUMSTSR, and performing the above techniques with the resulting protein or a peptide thereof
25 as an immunogen.

5. Pharmaceutical Composition and Cells of the Present Invention

The pharmaceutical composition of the present invention for the use as an AIDS onset inhibitor or an HIV-1 infection inhibitor comprises a murine PBSF/SDF-1.

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10 The pharmaceutical composition of the present invention can be administered orally or parenterally. In other words, the pharmaceutical composition can be orally administered in a form which is usually used for administration, including, for instance, tablets, capsules, granules, powder, and the like, or alternatively the pharmaceutical composition can be injected intramuscularly or subcutaneously in the form of liquid, emulsion, suspension, liposome, and the like. In
15 addition, the pharmaceutical composition can be administered to rectum as a suppository. These preparations can be produced by formulating the effective ingredients of the present invention with usual carriers, excipients, binding agents, stabilizers, buffers,
20 dissolution auxiliaries, isotonic agents, and the like, which are pharmaceutically acceptable.

The dosage and the number of administration may differ depending on symptoms, case history, ages, body weights, forms of administration, and the like of the
25 patients. For example, when orally administered to an

adult, it can be administered at once or divided in several portions by appropriately adjusting the dosage to a range of usually from 5 to 500 mg, preferably from 10 to 100 mg, per one day.

5 In addition, the cells of the present invention are cells expressing the polypeptide of the present invention described above, or cells expressing both of the above polypeptide and a human CD4 protein.

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10 The above cells can be obtained, for instance, by the following method. Specifically, a vector into which a polynucleotide encoding a murine CXCR4 is incorporated is obtained. As the vector, any of those known per se, such as pEFBOS, pCAGGS, and pMX can be used. Thereafter, the vector described above into which the polynucleotide is
15 incorporated, is introduced into cells to be expressed, whereby obtaining the cells of the present invention. As the cells to be expressed, there can be included a cell line derived from a Chinese hamster ovary, CHO cells, a human colon cancer cell line, SW480 cells, a human
20 osteoblastsarcoma cell line, HOS cells, a human glioblastoma cell line, U87MG cells, and the like. In addition, a method of introducing a vector includes, for instance, a calcium phosphate method and methods using Lipofectin (GibcoBRL) and Lipofectamine (GibcoBRL).

25 Since a murine CXCR4 is found to be an HIV-1

coreceptor, the cells of the present invention can be used to screen AIDS onset inhibitors, HIV-1 infection inhibitors, and PBSF/SDF-1 agonists and antagonists, to detect an AIDS onset or an HIV-1 infection, and the like.

5

6. Screening Method of the Present Invention

The screening method of the present invention includes a method of screening AIDS onset inhibitors, HIV-1 infection inhibitors, and murine or human PBSF/SDF-1 agonists and antagonists. Concretely, the following methods are illustrated.

10

1) A method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, characterized in that the method comprises the steps of:

15

(a) mixing the cells of the present invention described above; a human T-cell-line-tropic HIV-1; and a substance to be screened, and incubating the resulting mixture; and

(b) analyzing localization of an HIV-1 in the cells.

20

2) A method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, characterized in that the method comprises the steps of:

(a) mixing the cells of the present invention described above; cells expressing an HIV-1 envelope protein; and a substance to be screened, and incubating the

25

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resulting mixture; and

(b) determining a level of the fusion of the above cells with the cells expressing an HIV-1 envelope protein.

3) A method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, or a PBSF/SDF-1 agonist or antagonist, characterized in that the method comprises the steps of:

(a) mixing the cells of the present invention described above; a murine or human PBSF/SDF-1; and a substance to be screened, and incubating the resulting mixture; and

(b) determining an intracellular calcium ion level and/or determining a binding activity of an expressed polypeptide with the murine or human PBSF/SDF-1.

In addition, as a human T-cell-line-tropic HIV-1, there can be included an HIV-1 IIIB strain (provided by Prof. Harada of Kumamoto Univ.) and an HIV-1 NL432 strain (provided by Prof. Adachi of Tokushima Univ.).

Embodiment 1)

It is more preferable that the step for analyzing the localization of an HIV-1 is carried out with a monoclonal antibody against a human T-cell-line-tropic HIV-1.

The method of analysis using the above monoclonal

antibody is not particularly limited, and includes any known usual method.

In addition, as the method for analyzing the localization of an HIV-1, the following enzyme method may also be employed.

Specifically, as "the cells of the present invention" used in this method, those preferably used are cells expressing a human CD4 protein and a coreceptor (for instance, SW480, U87MG, HOS, and the like) in which a gene for an enzyme (such as β -galactosidase, luciferase, or CAT) is introduced downstream of LTR, which is an expression promoter of an HIV-1 gene. When the cells are infected with an HIV-1, a tat protein, which is one kind of viral proteins, is expressed which in turn activates LTR. Accordingly, the infection level can be quantified by determining the enzymatic activity in the cell lysate.

Embodiment 2)

As the cells expressing an HIV-1 envelope protein, there can be included, for instance, ones in which an HIV-1 envelope protein gene is introduced into an HeLaS3. Further, ones in which, for instance, a β -galactosidase subunit (either one of α or ω) gene is additionally introduced are preferably used. In addition, as "the cells of the present invention," there can be preferably

used, for example, ones in which a human CD4 protein and a coreceptor are introduced into an NIH3T3; and ones in which a β -galactosidase subunit (either one of α or ω , and different from that introduced into the cells expressing an HIV-1 envelope protein) gene is additionally introduced. When the cells expressing an HIV-1 envelope protein and the cells of the present invention are subjected to cell fusion, β -galactosidase α -subunit and ω -subunit are associated to form an active β -galactosidase. Accordingly, the cell fusion level can be measured by mixing the cells of both parties, culturing them, and then determining the galactosidase activity in the cell lysate.

Embodiment 3)

When an activity for increasing an intracellular calcium ion level is observed as a result of the incubation in Step (a), it is possible that the substance to be screened is an agonist. When the binding between the substance to be screened and a receptor is observed even though no increase in the activity for intracellular calcium ion level is observed, it is possible that the substance to be screened is an antagonist. In addition, when there are influences in the activity for increasing an intracellular calcium ion level of a murine PBSF/SDF-1

and/or on a binding activity with a receptor, i.e., the activities are inhibited, it is possible that the substance to be screened is an antagonist. In addition, the above antagonist is exemplified by a hematopoietic stem cell liberator.

7. Detection Kit and Detection Method

The kit for detecting an AIDS onset or an HIV-1 infection of the present invention is characterized in that the kit comprises the cells of the present invention.

By using the above kit, an AIDS onset or an HIV-1 infection can readily be detected. The kit of the present invention is intended to perform the detection using the method for detection of the present invention described below.

The method for detecting an AIDS onset or an HIV-1 infection of the present invention is characterized in that the method comprises;

- (a) mixing the cells of the present invention described above with sera, blood cells or blood of a patient suspected to be infected with an HIV-1, and incubating the resulting mixture, and
- (b) analyzing localization of an HIV-1 in the cells or determining a level of the fusion of the cells with HIV-1-infected cells.

As the method for analyzing localization of an HIV-1 used herein, there are included ones employed in an AIDS onset inhibitor or an HIV-1 infection inhibitor of the present invention. In addition, as the method of determining a level of the fusion of the cells with HIV-1-infected cells, there are included ones employed in an AIDS onset inhibitor or an HIV-1 infection inhibitor of the present invention. Incidentally, the term "HIV-1-infected cells" in Step (b) refers to HIV-1-infected blood cells of a patient suspected to be infected with an HIV-1.

8. Utility of the Present Invention

Both of a murine CXCR4 and a human CXCR4/fusin/HUMSTSR of the present invention react with a murine PBSF/SDF-1. Since there is a difference of only one amino acid out of 71 amino acids in the murine and human PBSF/SDF-1s, a murine CXCR4 is expected to be bound also with a human PBSF/SDF-1. Since a human PBSF/SDF-1 inhibits an infection with a T-cell-line-tropic HIV-1 mediated by a CXCR4/fusin/HUMSTSR, an antibody against the murine CXCR4 protein of the present invention and an antibody against a chimera protein having a binding site with a T-cell-line-tropic HIV-1 resulting from mutual substitution of the extracellular domains of a murine

CXCR4 and a human CXCR4/fusin/HUMSTR can be used as an HIV-1 infection inhibitor, i.e., a therapeutic agent against AIDS.

By means of the method of screening agonists and antagonists of a chimera protein resulting from mutual substitution of the extracellular domains of a murine CXCR4 protein, and murine CXCR4 and human CXCR4/fusin/HUMSTR provided by the present invention, such agonists and antagonists can be obtained, each of which can be used as an HIV-1 infection inhibitor, i.e., a therapeutic agent against AIDS.

The present invention will be described in further detail by means of the following working examples, but the present invention is by no means limited to these examples.

Example 1 (Cloning of cDNA of Murine CXCR4)

(1) Synthesis of Primer

Based on a known amino acid sequence of a chemokine receptor, a condensed forward primer C2F2-2 (SEQ ID NO: 5 of Sequence Listing) to a DNA sequence encoding an amino acid sequence of a second transmembrane-spanning domain, and a condensed reverse primer C4R1 (SEQ ID NO: 6 of

Sequence Listing) to a DNA sequence encoding an amino acid sequence of a seventh transmembrane-spanning domain were synthesized using a DNA synthesizer ("Cyclone Plus," Nippon Millipore).

5

(2) Purification of mRNA from Murine Pre-B-Cell Line
DW34

10 A murine pre-B-cell line DW34 was suspended in RPMI 1640 medium. After culturing for one week, the culture was washed with Dulbecco PBS(-) [Nissui], and mRNA was purified by using mRNA Purification Kit (Pharmacia).

(3) Cloning of cDNA Fragment of Murine CXCR4

15 A single-stranded cDNA was synthesized from 200 ng of mRNA purified from a murine pre-B-cell line DW34 with Ready-To-Go T-Primed First-Strand Kit (Pharmacia). PCR reaction (30 cycles under conditions of 94°C for 0.5 minutes, 55°C for 0.5 minutes, and 72°C for 1 minute) was carried out using the resulting single-stranded cDNA as a template, C2F2-2 and C4R1 as primers, and Taq as a thermostable DNA polymerase. The resulting reaction mixture was separated by low-melting point agarose gel electrophoresis, and a DNA band of a desired size (about 690 bp) was excised, and a DNA fragment was purified by
25 using Wizard PCR Preps DNA Purification System (Promega).

5 The resulting DNA fragment was inserted into pT7Blue
vector by using a DNA Ligation Kit (Takara). The
nucleotide sequence of the inserted DNA was determined by
PRISM Ready Reaction Sequence Kit (Applied Biosystems) and
DNA Sequencer (Applied Biosystems). The resulting cDNA
sequence of a murine CXCR4 is shown by SEQ ID NO: 2 of
Sequence Listing. Based on the cDNA sequence of a murine
CXCR4 obtained in the manner described above, primers as
shown by SEQ ID NO: 7 of Sequence Listing and as shown by
10 SEQ ID NO: 8 of Sequence Listing were synthesized, and a
cDNA clone containing 5'-terminal was obtained using the
cDNA of DW34 cells obtained as described above as a
template, with Marathon cDNA Amplification Kit (Clontech).
The resulting cDNA sequence of a murine CXCR4 is shown by
15 SEQ ID NO: 3 of Sequence Listing.

Example 2 (Expression of Murine CXCR4 in Each Tissue)

(1) Preparation of Probe

20 In order to study the expression of a murine CXCR4 in
each murine tissue, firstly, a probe was prepared as
follows. Based on the nucleotide sequence of a murine
CXCR4 gene, a DNA sequence (SEQ ID NO: 15 of Sequence
Listing) in the same direction corresponding to the second
25 transmembrane-spanning-domain portion, and a DNA sequence

(SEQ ID NO: 16 of Sequence Listing) in the opposite direction corresponding to the seventh transmembrane-spanning-domain portion were synthesized as primers to be used in the subsequent PCR. PCR reaction was carried out for 30 cycles under conditions of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes, using the cDNA of the nucleotide sequence obtained in Item (3) of Example 1 above as a template, and a Taq Polymerase. The resulting reaction mixture of the PCR was separated by agarose gel electrophoresis, and a DNA band of a desired size (about 690 bp) was excised, and a DNA fragment was purified by using Wizard PCR Preps DNA Purification System. Fifty nanograms of the resulting DNA fragment was ³²P-labeled by using Prime-It II Random Primer Labelling Kit (Stratagene) to be used as a probe.

(2) Northern Blotting Analysis of Murine Tissue and Murine Fetus

mRNAs Of various murine tissues and mRNAs of murine fetuses of 7 days, 11 days, 15 days and 17 days after embryogenesis were separated by electrophoresis, and the transferred membrane and the probe obtained in Item (1) above were subjected to hybridization. The membrane was washed by immersing in 2 × SSC containing 0.05% SDS at room temperature for 15 minutes twice, and further

immersing in 0.1 × SSC containing 0.1% SDS at 50°C for
20 minutes twice. The radioactive rays of this membrane
were detected by autoradiography. The results are shown
in Figure 2 A (murine tissue) and B (murine fetus). It is
5 clear from the intensities of the bands that strong
signals were obtained at thymus, lymph node, and spleen,
and weak signals were obtained at brain, small intestine,
stomach, and kidney. In addition, strong signals were
obtained in the entire murine fetus.

10 Example 3 (Cloning of Genomic DNA of Murine CXCR4)

(A) Preparation of Probe

Based on the nucleotide sequence of a murine CXCR4
15 cDNA obtained in Item (3) of Example 1 above, appropriate
forward and reverse primers were synthesized to be used in
the subsequent PCR. A double-stranded DNA was obtained
from the cDNA in Item (3) of Example 1, and PCR reaction
was carried out using this double-stranded DNA as a
20 template and a Taq polymerase. The reaction product was
separated by agarose gel electrophoresis, and a DNA band
of a desired size (about 690 bp) was excised, and a DNA
fragment was purified. Fifty nanograms of the resulting
DNA fragment was ³²P-labeled using Prime-It II Random
25 Primer Labelling Kit (Stratagene) as a probe.

(B) Cloning of Murine Genomic Library

First, with a 129/SvJ murine liver genomic library incorporated into a phage vector λ FIXII was infected *Escherichia coli*, and as a primary screening, and spread over a plate to form a plaque, and the plaque was transferred to nylon membrane (Du Pont). This membrane was pre-hybridized by immersing the membrane in a pre-hybridization reagent [5 × SSPE (0.9M NaCl, 0.05M sodium phosphate at pH 7.7, 0.005M Na₂EDTA), 50% formamide, 5 × Denhardt's reagent, 50 µg/ml salmon sperm DNA, 0.1% SDS]. Thereafter, the pre-hybridized membrane was hybridized together with the probe obtained in Item (A) above at 42°C for 15 hours by immersing the membrane in a hybridization reagent [5 × SSPE, 50% formamide, 1 × Denhardt's reagent, 10%-dextran disodium sulfate, 50 µg/ml salmon sperm DNA, 0.1% SDS]. After washing the membrane, the radioactivity was detected, and positive plaques giving signals were selected. The selected plaques were successively diluted to carry out secondary and tertiary screening, and whereby two single clones were selected.

The cloned phage DNA was cleaved with various restriction enzymes, and separated by agarose gel electrophoresis. Those bands having the same patterns were considered to be the same clone, and the cloned phage

DNA was cleaved such that a positive band of the size as small as possible could be obtained by repeating the same hybridization. The selected positive DNA fragment was inserted into a pBluescripts KSII vector, and the nucleotide sequence was determined by dideoxy method. The resulting DNA sequence of the murine CXCR4 gene is shown by SEQ ID NO: 4 of Sequence Listing. A nucleotide sequence containing the longest open reading frame was found from the nucleotide sequence as shown by SEQ ID NO: 3 of Sequence Listing and the nucleotide sequence as shown by SEQ ID NO: 4 of Sequence Listing, and its nucleotide sequence is shown by SEQ ID NO: 1 of Sequence Listing. Also, this nucleotide sequence as shown by SEQ ID NO: 1 of Sequence Listing was subjected to nucleic acid homology search with the GenBank/EMBL/DDBJ DNA sequence data base. As a result of the search, it was clarified that the resulting clone is a DNA encoding a novel murine chemokine receptor, and the clone was named murine CXCR4.

Example 4 (Homology Analysis of Amino Acid Sequence of Murine CXCR4)

The amino acid sequence (SEQ ID NO: 17 of Sequence Listing) which was deduced based on the nucleotide sequence of a murine CXCR4 was estimated to be a trimer G

protein-coupled receptor containing a seven transmembrane-
spanning domain, characteristic to a chemokine receptor.
The amino acid sequence thereof was compared with a known
sequence of a CXC chemokine receptor (GenBank, EMBL, DDBJ
5 were used as data base, and analyzed with BLAST). As a
result, the analyzed sequence most closely resembled human
CXCR4/fusin/HUMSTSR (90% identity), and homologies with
monkey CXCR4 and bovine CXCR4 were 89% and 86%,
respectively, and homologies with rat IL-8RB, rabbit
10 IL-8RA, and rabbit IL-8RB were 49%, 47%, and 45%,
respectively.

Example 5 (Expression of Murine CXCR4 and Human
CXCR4/fusin/HUMSTSR)

- 15 (1) Preparation for Expression Vectors of Murine CXCR4,
Human CCCRR2B and Human CXCR4/fusin/HUMSTSR

In order to clone previously reported human chemokine
receptors a CC CKR2B gene and a CXCR4/fusin/HUMSTSR gene,
20 PCR reaction was carried out in the following manner using
a cDNA of a human monocyte cell line THP-1. Five-hundred
nanograms of a cDNA of the THP-1 cells was used as a
template, the primers as shown by SEQ ID NO: 11 and
SEQ ID NO: 12 of Sequence Listing were used for
25 amplification of human CXCR4/fusin/HUMSTSR, and the

primers as shown by SEQ ID NO: 9 of Sequence Listing and
SEQ ID NO: 10 of Sequence Listing were used for
amplification of CC CKR2B, each primer of which was used
in an amount of 500 ng. As the enzyme for the reaction,
5 Taq Polymerase (Takara Shuzo) was used. The reaction was
carried out for 1 cycle at 94°C for 3 minutes; thereafter,
35 cycles at 94°C for 1 minute, 55°C for 2 minutes, and
72°C for 3 minutes; and further at 72°C for 3 minutes.
The gene fragments of human CXCR4/fusin/HUMSTSR and
10 CC CKR2B obtained by the reaction were each incorporated
into TA cloning sites of pCRII (Invitrogen). The plasmids
obtained in the manner described above were named
pCRIICXCR4 and pCRIICC CKR2B, respectively. Subsequently,
the resulting pCRIICXCR4 and pCRIICC CKR2B plasmids were
15 respectively digested with NotI and XboI (both from Takara
Shuzo), and the digested fragment was incorporated into a
NotI/XboI site of pCAGGStkNeo. The plasmids obtained in
the manner described above were named pCANCXCR4 and pCANCC
CKR2B, respectively.

20 In order to clone the murine CXCR4 gene, PCR
technique was carried out using a single-stranded cDNA of
the murine pre-B-cell line DW34 as shown by SEQ ID NO: 3
of Sequence Listing obtained in Item (3) of Example 1
above as a template. One hundred nanograms of the cDNA
25 was used as a template, and the primers as shown by

SEQ ID NO: 13 of Sequence Listing and as shown by
SEQ ID NO: 14 of Sequence Listing were used. As the
enzyme used for reaction, ExTaq (Takara Shuzo) was used.
The reaction was carried out for 1 cycle at 94°C for
3 minutes; thereafter, 20 cycles at 94°C for 1 minute,
55°C for 1 minute, and 72°C for 2 minutes; and further at
72°C for 5 minutes. The resulting murine CXCR4 gene
fragment was digested with NotI and XhoI (Takara Shuzo),
and the digested fragment was incorporated into a
NotI/XhoI site of pCAGGStkNeo. The plasmid obtained in
the manner described above was named pCANmPBSFR.

(2) Expression of Murine CXCR4, Human CXCR4/fusin/HUMSTSR
and Human CC CKR2B in CHO Cells

CHO cells were cultured in a cell culture petri dish
(Iwaki Glass Co., Ltd.) having a diameter of 10 cm at 37°C
for 1 day in the presence of 10% CO₂ gas. Each 30 µg of
DNAs of expression vectors of the three chemokine
receptors obtained in Item (1) above (pCANmPBSFR,
pCANCXCR4 and pCANCC CKR2B) was dissolved in 25 µl of
distilled water, and to the resulting mixed solution was
added 500 µl of 250 mM calcium chloride (nacalaitesque).
To the liquid mixture of the DNA and calcium chloride was
added 500 µl of 2 × BBS solution [50 mM BES (SIGMA),
280 mM sodium chloride (nacalaitesque) and 1.5 mM disodium

hydrogenphosphate (nacalaitesque)], and then allowed to stand at room temperature for 25 minutes. The DNA solution prepared in the manner described above was added dropwise to the petri dish in which the CHO cells were cultured, and the cells were cultured at 35°C for 20 hours in the presence of 3% CO₂ gas to introduce the DNA into the cells. The cells into which the DNA was introduced were washed twice with 3 ml of PBS(+), and thereto was added 10 ml of α-MEM (GIBCO) containing 10% FCS solution, and the cells were cultured at 37°C for one day in the presence of 5% CO₂ gas.

Subsequently, the cells were suspended in a medium prepared by adding 2 mg/ml GENETICIN (Wako Pure Chemical Industries, Ltd.) to an α-MEM (GIBCO) medium containing 10% FCS, and divided into cell culture petri dishes (Iwaki Glass Co., Ltd.) each having a diameter of 10 cm at a cell density of 5×10^3 cells/petri dish. The culture was continued at 37°C in the presence of 10% CO₂ gas, and GENETICIN-resistant cells were used for determination of an intracellular calcium level as CHO cell lines expressing a murine CXCR4, a human CXCR4/fusin/HUMSTSR, and CC CKR2B. As illustrated in Example 6 given below, since the CC CKR2B was found to have an activity of increasing the intracellular calcium level owing to the addition of a specific ligand MCP-1, the expression of the

receptor was confirmed. In addition, the murine CXCR4 and the human CXCR4/fusin/HUMSTSR were also considered to be similarly expressed, because transformation and culture were carried out in the same manner using the same cell line as the CC CKR2B.

Example 6 (Biological Activity of Murine CXCR4)

Each of the CHO cells expressing the murine CXCR4 and the human chemokine receptors (CXCR4/fusin/HUMSTSR and CC CKR2B) obtained in Item (2) of Example 5 above was washed with Dulbecco PBS(-), and then suspended in an HBSS buffer (containing 125 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 0.5 mM glucose and 0.1% BSA in 20 mM Hepes at pH 7.4) at a cell density of 5×10^6 cells/ml. To the resulting suspension was further added fura-PE3AM (Texas Fluorescence Laboratory) so as to have a concentration of 2.5 μ M, and the cells were then incubated at 37°C for 30 minutes. After washing with the HBSS buffer, each of the cells expressing CC chemokine receptor was suspended in the same buffer at a cell density of 5×10^6 cells/ml. The changes in the fluorescence when each chemokine (murine PBSF/SDF-1 or human MCP-1) was added to 500 μ l of each of the resulting suspensions of the cells expressing CC chemokine receptor so as to have a concentration of 100 nM were determined with a spectrofluorophotometer

(LS50B, PERKIN-ELMER) at the excitation wavelengths of 340 nm and 380 nm, the fluorescence wavelength of 510 nm and the response of 0.5 seconds. The results are shown in Figure 3 to 6 which are represented in terms of the ratio of the fluorescence intensity at 340 nm and that at 380 nm over a passage of a time period.

In the murine PBSF/SDF-1 stimulation, there was found to be an increase in the ratios of fluorescence intensity in the cells expressing a murine CXCR4 and a human CXCR4/fusin/HUMSTR, while there was found to be no increase in the ratios of fluorescence intensities in the cells expressing CC CKR2B, which is a CC chemokine receptor. Incidentally, in the stimulation with a MCP-1 peptide, which is a positive control to a receptor, there was found to be an increase in the ratio of fluorescence intensities in the cells expressing CC CKR2B. Therefore, the murine PBSF/SDF-1 was found to have an activity of increasing the intracellular calcium ion level specifically to the CHO cells expressing the murine CXCR4 and the human CXCR4/fusin/HUMSTR according to the present invention. Also, as shown in Figure 7, in the cells expressing a murine CXCR4, the desensitization was found wherein there were no changes in the ratio of the fluorescence intensities by continuous addition of a murine PBSF/SDF-1. The desensitization was not found when a human MCP-1, a

negative control, was added. From these results, it was clarified that the receptor of the present invention is the receptor of a murine PBSF/SDF-1 receptor.

5 Example 7

Materials and Methods

Cell lines: Murine NIH3T3 cells, SW480 cells derived from human small intestine epithelium, and U87MG derived from human gliocyte were cultured in DMEM containing 10% FCS. Human HeLaS3 cells were cultured in RPMI1640 containing 10% FCS. HOS cells derived from human osteoblasts were cultured in Eagle MEM containing 1% non-essential amino acids (Gibco) and 10% FCS.

Viruses: An HIV-1 NL432 strain was provided by Prof. Adachi (Tokushima Univ.). A IIIB strain was provided by Prof. Harada (Kumamoto Univ.). An SF162 strain was provided by Prof. J. A. Levy (San Francisco Univ., California). HIV-1 chimera virus clones, NL432env-162 and NL432V3-162 were provided by Isaka (Shionogi & Co., Ltd.). Recombinant vaccinia viruses, Vac. Env (NL432 env), Vac. Env 162 (SF162 env) and Vac T4 (CD4) were provided by Prof. Shioda (Tokyo Univ.). LO-T7 (T7 polymerase) was provided by M. Kohara (TORITSU RINSHOKEN).

Transfection to cells: NIH3T3 cells were cultured overnight in a 24-well plate at a cell density of 5×10^4 cells per well, and transfected with a chemokine receptor gene which was incorporated into pBluescript using Lipofectamine (Gibco). 4 Hours after initiation of the transfection, the cells were washed with PBS, and thereto a culture medium was added, and then cultured at 37°C overnight to be subjected to a fusion assay. The SW480 cells and the HOS cells were cultured overnight in a 6 cm-plate at a cell density of 5×10^5 cells. The SW480 cells were transfected with a plasmid mixture of 5 µg of the receptor gene which was incorporated into pEF-BOS, 7.5 µg of T4-Neo, a vector expressing CD4, and 2.5 µg of LTR (EcoRV)-β-Gal-Neo by means of a modified calcium phosphate method. The HOS cells constitutively expressing a human CD4 and an LTR-Gal were transfected with 15 µg of a receptor gene which was incorporated into pEF-BOS by the same method. Each of the cells was cultured at 35°C overnight in the presence of 3% CO₂, washed with PBS(-), and collected with PBS containing 0.5 mM EDTA. Thereafter, the culture was plated in over a 12-well plate, and cultured at 37°C overnight to be subjected to an infection assay.

Cell fusion assay: In order to quantify the cell

fusion, we employed a modified cell fusion assay utilizing α -complementation of β -galactosidase (β -gal) (Shida et al., manuscript in preparation).

A β -gal α -subunit and an env protein were introduced into HeLaS3 cells (24-well plate, 1×10^5 cells/well), which are effector cells, by using a recombinant vaccinia virus.

A human CD4, a β -gal ω -subunit, and a T7 RNA polymerase were introduced into NIH3T3 cells (24-well plate, 5×10^4 cells/well), which are target cells, by using a recombinant vaccinia virus, and a chemokine receptor was transfected to the target cells using Lipofectamine. 16 Hours after transfection, the effector cells and the target cells were washed with PBS containing 0.5 mM CaCl_2 , and treated with 2D5, an anti-vaccinia virus antibody, in order to inhibit the non-specific cell fusion caused by the vaccinia virus. The effector cells were suspended in a Hanks buffer (pH 7.6) containing 3 mM CaCl_2 , and overlaid on the target cells in the 24-well plate. Thereafter, the resulting overlaid mixture was centrifuged at 1,300 rpm for 5 minutes to initiate the cell fusion.

After centrifugation, the cells were cultured at 37°C for 12 hours in the presence of 5% CO_2 . When the cell fusion takes place, α -subunit and ω -subunit of the β -gal

contained in the cytoplasmas of the fusion cells are associated with each other to form an active β -gal enzyme by α -complementation. Therefore, after the culture medium was removed, a solution containing 8 mM

5 chlorophenolred-b-D-galactopyranoside (Boehringer Mannheim), which is a β -gal substrate, 45 mM 2-mercaptoethanol, 1 mM $MgCl_2$, 100 mM Hepes at pH 8.0, 0.5% NP40 and 0.1 mg/ml DNase I was added in an amount of 200 μ l per one well, and reacted at 37°C for 30 minutes. 10 Thereafter, 2% SDS was added in an amount of 200 μ l per one well to terminate the reaction. In order to quantify the β -gal activity in the reaction mixture, the absorbance was measured at a wavelength of 590 nm.

15 Infection assay: The human SW480 or HOS cells each expressing a human CD4 and a receptor were cultured in a 12-well plate. To each well was added a culture containing an HIV-1 virus (reverse transcriptase (RT) activity: SF162: 2×10^6 RT/mL; NL432env162, NL432V3-162 20 and IIIB: 5×10^6 RT/mL; NL432: 3×10^6 RT/mL), and cultured at 37°C for 2 hours in the presence of 5% CO_2 , and then the culture medium was added in an amount of 2.5 mL per one well. 4 Days after infection, a Reporter lysis buffer (Promega) was added in an amount of 400 μ l per one 25 well, frozen at -80°C, and then thawed. The thawed sample

was transferred to an Eppendorf tube, and centrifuged at 12,000 rpm at 4°C for 5 minutes. Thereafter, the β -gal activity contained in the supernatant was measured with a luminescence β -gal detection kit (Clontech).

5

Results

First, in order to study whether or not a murine CXCR4 supports an env-mediated cell fusion of an HIV-1, we conducted an experiment using an assay system in which β -Gal is activated by the fusion of the effector cells (HeLaS3 cells) expressing an env protein with the target cells (NIH3T3 cells) expressing a human CD4 and a receptor. In this assay, the HeLaS3 cells, effector cells, were subjected to infection with a recombinant vaccinia virus to express an α -subunit of the β -Gal and an HIV-1 env protein, and the NIH3T3 cells, target cells, were subjected to infection with a recombinant vaccinia virus to express an ω -subunit of the β -Gal, a T7 polymerase and a human CD4. After infection with a virus, the NIH3T3 cells were further transfected with a plasmid carrying a human CXCR4, a human CCR5, or a murine CXCR4. After an overnight culture, the effector cells and the target cells were mixed and cultured. When the cell fusion takes place, the α -subunit and the ω -subunit of the β -Gal contained in the cytoplasmas of the fusion cells are

associated with each other, whereby activating the β -Gal. As shown in Figure 8, the HeLaS3 cells expressing an env protein derived from NL432, a T-cell-line-tropic HIV-1, are fused with the NIH3T3 cells expressing a human CXCR4 and a human CD4, but not with the NIH3T3 cells expressing a human CCR5 and a human CD4.

Surprisingly, the HeLaS3 cells expressing an env protein derived from NL432 are also fused with the cells expressing a murine CXCR4 and a human CD4 similarly. The HeLaS3 cells expressing an env protein derived from SF162, a monocyte-tropic HIV-1, are fused with the NIH3T3 cells expressing a human CCR5 and a human CD4, but not with the NIH3T3 cells expressing a human CXCR4 or a murine CXCR4 and a human CD4.

Secondly, we have studied whether cells which expressed a murine CXCR4 were infected with a virus. Since murine cells which expressed human CXCR4 and CD4, including NIH3T3 cells, had a low HIV-1 replication efficiency, three human cell lines, namely, the SW480 cells derived from human small intestine epithelium, the HOS cells derived from osteoblasts, and the U87MG cells derived from human gliocytes were used as the target cells for viral infection. These cells were transfected with a reporter gene (lacZ) having an LTR of an HIV-1 as a promoter. When the cells are infected with a virus, Tat

protein, which is a transcription-activating factor derived from an HIV-1, is expressed to act on an LTR, whereby inducing the expression of lacZ. These cells were further transfected with a human CD4 and a chemokine receptor, and then subjected to infection with a T-cell-line-tropic virus strain (NL432, IIIB) or a monocyte-tropic virus strain (SF162). As shown in Figure 9, with NL432 and IIIB were infected similarly both of SW480 expressing a murine CXCR4 and a human CD4, and SW480 expressing a human CXCR4 and a human CD4. These findings were consistent with the results of the fusion assay described above. On the contrary, when the human CCR2b or CCR5 was expressed instead of CXCR4, the cells were not infected with these viruses.

On the other hand, with SF162, the SW480 expressing a human CCR5 and a human CD4 was infected, but cells expressing a murine CXCR4 and a human CD4, and cells expressing a human CXCR4 and a human CD4 were not infected therewith. In addition, similar results were obtained even when the HOS cells or the U87MG cells were used instead of the SW480 cells (Figure 10 and Figure 11). Thus, it was suggested that a murine CXCR4 supports the entry of a T-cell-line-tropic HIV-1 into target cells, and that it does not affect the DNA synthesis of a provirus, the integration to a genomic DNA or the viral expression

in human cells.

Incidentally, there was clarified that the HIV-1 entry mediated by a human CXCR4 was inhibited by a monoclonal antibody against the V3 loop of an env protein.

5 Accordingly, we have studied whether the V3 loop of the env protein (gp120) (of a T-cell-line-tropic virus strain) is also required for a murine CXCR4-mediated HIV-1 entry in order to confirm the function of a murine CXCR4 is similar to that of a human CXCR4. For this purpose, the
10 SW480 cells expressing a human CD4 and a chemokine receptor were subjected to infection with NL432env-162 or NL432V3-162 which is the chimera virus clone of NL432 and SF162. As shown in Figure 12, NL432env-162 is a provirus clone in which the env region of a T-cell-line-tropic
15 virus strain NL432 is substituted with that of a monocyte-tropic HIV-1 strain SF162, and NL432V3-162 is a provirus clone in which the V3 loop of the env of NL432 is substituted with that of SF162. The SW480 cells
20 expressing a murine CXCR4 and a human CD4 were infected with NL432, but these cells were not infected with NL432env-162 or NL432V3-162 (Figure 13).

On the other hand, the SW480 cells expressing a human CCR5 and a human CD4 were infected with NL432env-162 and with NL432V3-162 (Figure 13). It was clarified from the
25 results that in the case of a murine CXCR4, a V3 loop of

an NL432 env is also required for the viral entry in the same manner as the human CXCR4.

Discussion

5 From the studies described above, there has been clarified that the murine CXCR4 supports the cell membrane fusion mediated by a T-cell-line-tropic HIV-1 env and the infection with a T-cell-line-tropic HIV-1. These results suggest that the murine CXCR4 is not a species-specific
10 barrier against the infection with an HIV-1. In the existing studies, there has been clarified even if a human CD4 were expressed in a cell line from murine lymphocytes or non-lymphocytes such as NIH3T3 or T-cell clone 3DT, the HIV-1 is adsorbed but not allowed to enter. One of the
15 interpretations of these results is that the CXCR4 is not expressed on the surface of the murine cells which have expressed a human CD4. In fact, a murine PBSF/SDF-1 stimulation does not induce the change in the intracellular calcium level in the NIH3T3 cells.
20 Nevertheless, the murine CXCR4 is expressed in thymocytes in which both of CD4 and CD8 are positive as well as in thymocytes in which either CD4 or CD8 is positive. Therefore, it is important (in order to invalidate the interpretation described above) to determine whether or
25 not the 3DT cells used in the experiment express CXCR4.

In a recent study, there has been clarified that a murine homologue (murine CCR5) of a human CCR5, which is a receptor for a monocyte-tropic HIV-1, gives no support to an HIV-1 entry. This result suggests that there is a difference in the species-specificity between a receptor for a monocyte-tropic HIV-1 and a receptor for a T-cell-line-tropic HIV-1. This difference may be due to the fact that an amino acid sequence of CXCR4 is highly conserved in between the species, as compared to those of other chemokine receptors including CCR5. The amino acid sequence of a murine CXCR4 has a 90% identity with that of a human CXCR4, while a CCR5 and a CXCR2 exhibit only 82% and 71% identity, respectively, between the murine and human sequences. This inter-species high conservation of CXCR4 reflects the fact that PBSF/SDF-1, which is a CXCR4 ligand, has a unique function as compared to other chemokines such as MIP-1 α , MIP- β and RANTES, which are CCR5 ligands. In contrary to the understanding that the chemokines other than PBSF/SDF-1 are involved in the chemotaxis of leukocytes in inflammation, PBSF/SDF-1 has a function essential for a biological development, such as hematopoiesis and cardiogenesis.

From the existing study results and from the facts that the production efficiency of virus particles is low as compared to that of human cells, even though a murine

cell line NIH3T3 expressing a human CD4 and a chemokine
receptor supports an HIV-1 entry, the murine cells may
lack some intracellular molecules required for the
replication of an HIV-1. However, an HIV-1-infected model
5 mouse will be developed by generation of a transgenic
mouse in which a human gene, which is a molecule causing a
species-specific barrier, is introduced. Our results
revealed the fact that it is not necessary to introduce a
human CXCR4 gene into an HIV-1-infected model mouse. In
10 addition, since an *in vivo* expression of CXCR4 is more apt
to study the initiation and the progress of the transition
from a monocyte-tropic HIV-1 to a T-cell-line-tropic HIV-1
which leads to an onset of AIDS, there is provided
valuable information for the development of an animal
15 model for simulation of an entire process of an HIV-1
infection.

INDUSTRIAL APPLICABILITY

According to the present invention, there can be
20 provided a novel murine CXC chemokine receptor gene; a
polypeptide encoded by the gene; an expression vector
carrying the gene; a transformant harboring the expression
vector; a monoclonal antibody against the polypeptide; a
method for producing the polypeptide using the
25 transformant; and a method of screening an agonist or

5

SEQUENCE LISTING

SEQ ID NO: 1

SEQUENCE LENGTH: 1877

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE

ORGANISM: mouse

SEQUENCE DESCRIPTION:

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GGGCTCGAGC GGCCGCCCCG GCAGGTGCAG GTAGCAGTGA CCCTCTGA 71

GGCGTTTGGTGCTCCGGTAACCAACACGGCTGTAGAGCGAGTGTTGCC 119

ATGGAACCGATCAGTGTGAGTATATACACTTCTGATAACTACTCTGAA 167

MetGluProIleSerValSerIleTyrThrSerAspAsnTyrSerGlu

1 5 10 15

GAAGTGGGGTCTGGAGACTATGACTCCAACAAGGAACCCTGCTTCCGG 215

GluValGlySerGlyAspTyrAspSerAsnLysGluProCysPheArg

20 25 30

GATGAAAACGTCCATTTCAATAGGATCTTCCTGCCCACCATCTACTTC 263

AspGluAsnValHisPheAsnArgIlePheLeuProThrIleTyrPhe

35 40 45

ATCATCTTCTTGACTGGCATAGTCGGCAATGGATTGGTGATCCTGGTC 311

IleIlePheLeuThrGlyIleValGlyAsnGlyLeuValIleLeuVal

50 55 60

ATGGGTACCAGAAGAAGCTAAGGAGCATGACGGACAAGTACCGGCTG 359

MetGlyTyrGlnLysLysLeuArgSerMetThrAspLysTyrArgLeu

65 70 75 80

CACCTGTCAGTGGCTGACCTCCTCTTTGTCATCACACTCCCCTTCTGG 407

HisLeuSerValAlaAspLeuLeuPheValIleThrLeuProPheTrp

85 90 95

GCAGTTGATGCCATGGCTGACTGGTACTTTGGGAAATTTTGTGTAAG 455

AlaValAspAlaMetAlaAspTrpTyrPheGlyLysPheLeuCysLys

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	115	120	125	
CTGGCCTTCATCAGCCTGGACCGGTACCTCGCCATTGTCCACGCCACC				551
LeuAlaPheIleSerLeuAspArgTyrLeuAlaIleValHisAlaThr				
	130	135	140	
AACAGTCAAAGGCCAAGGAAACTGCTGGCTGAAAAGGCAGTCTATGTG				599
AsnSerGlnArgProArgLysLeuLeuAlaGluLysAlaValTyrVal				
	145	150	155	160
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GlyValTrpIleProAlaLeuLeuLeuThrIleProAspPheIlePhe				
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AlaAspValSerGlnGlyAspIleSerGlnGlyAspAspArgTyrIle				
	180	185	190	
TGTGACCGCCTTTACCCCGATAGCCTGTGGATGGTGGTGTTC AATTC				743
CysAspArgLeuTyrProAspSerLeuTrpMetValValPheGlnPhe				
	195	200	205	
CAGCATATAATGGTGGGTCTCATCCTGCCCGGCATCGTCATCCTCTCC				791
GlnHisIleMetValGlyLeuIleLeuProGlyIleValIleLeuSer				
	210	215	220	
TGTTACTGCATCATCATCTCTAAGCTGTACACTCCAAGGGCCACCAG				839
CysTyrCysIleIleIleSerLysLeuSerHisSerLysGlyHisGln				
	225	230	235	240
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LysArgLysAlaLeuLysThrThrValIleLeuIleLeuAlaPhePhe				
	245	250	255	
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	260	265	270	
CTTTTGGGAGTCATCAAGCAAGGATGTGACTTCGAGAGCATTGTGCAC				983
LeuLeuGlyValIleLysGlnGlyCysAspPheGluSerIleValHis				
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```
SEQ ID NO: 2
SEQUENCE LENGTH: 690
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: double
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STRANDEDNESS: double

ORIGINAL SOURCE

SEQUENCE DESCRIPTION:

3

1

HisLeuSerValAlaAspLeuLeuPheValIleThrLeuProPheTrp

15

AlaValAspAlaMetAlaAspTrpTyrPheGlyLysPheLeuCysLys

30

AlaValHisIleIleTyrThrValAsnLeuTyrSerSerValLeuIle

45

LeuAlaPheIleSerLeuAspArgTyrLeuAlaIleValHisAlaThr

65

AsnSerGlnArgProArgLysLeuLeuAlaGluLysAlaValTyrVal

80

GlyValTrpIleProAlaLeuLeuLeuThrIleProAspPheIlePhe

95

AlaAspValSerGlnGlyAspIleSerGlnGlyAspAspArgTyrIle

110

CysAspArgLeuTyrProAspSerLeuTrpMetValValPheGlnPhe

125

GlnHisIleMetValGlyLeuIleLeuProGlyIleValIleLeuSer

- 70 -

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CysTyrCysIleIleIleSerLysLeuSerHisSerLysGlyHisGln
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AAGCGCAAGGCCCTCAAGACGACAGTCATCCTCATCCTAGCTTTCTTT 531
LysArgLysAlaLeuLysThrThrValIleLeuIleLeuAlaPhePhe
165 170 175
GCCTGCTGGCTGCCATATTATGTGGGGATCAGCATCGACTCCTTCATC 579
AlaCysTrpLeuProTyrTyrValGlyIleSerIleAspSerPheIle
180 185 190
CTTTTGGGAGTCATCAAGCAAGGATGTGACTTCGAGAGCATTTGTGCAC 627
LeuLeuGlyValIleLysGlnGlyCysAspPheGluSerIleValHis
195 200 205
AAGTGGATCTCCATCACAGAGGCCCTCGCCTTCTTCCACTGTTGCCTG 675
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210 215 220 225
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230

SEQ ID NO: 3

SEQUENCE LENGTH: 685

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE

ORGANISM: mouse

SEQUENCE DESCRIPTION:

CCATCCTAATACGACTCACTATA 23
GGGCTCGAGCGGCCCGCCCGGGCAGGTGCAGGTAGCAGTGACCCCTCTGA 71
GGCGTTTGGTGCTCCGGTAACCACCACGGCTGTAGAGCGAGTGTTGCC 119
ATGGAACCGATCAGTGTGAGTATATACACTTCTGATAACTACTCTGAA 167
MetGluProIleSerValSerIleTyrThrSerAspAsnTyrSerGlu

1 5 10 15
GAAGTGGGGTCTGGAGACTATGACTCCAACAAGGAACCCTGCTTCCGG 215
GluValGlySerGlyAspTyrAspSerAsnLysGluProCysPheArg
20 25 30
GATGAAAACGTCCATTTCAATAGGATCTTCCTGCCCACCATCTACTTC 263
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35 40 45
ATCATCTTCTTGACTGGCATAAGTCGGCAATGGATTGGTGATCCTGGTC 311
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50 55 60
ATGGGTTACCAGAAGAAGCTAAGGAGCATGACGGACAAGTACCGGCTG 359
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HisLeuSerValAlaAspLeuLeuPheValIleThrLeuProPheTrp
85 90 95
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115 120 125
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GGCGTCTGGATCCCAGCCCTCCTCCTGACTATACCTGACTTCATCTTT 647
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GCCGACGTCAGCCAGGGGGACATCAGTCAGGGGGATGA 685
AlaAspValSerGlnGlyAspIleSerGlnGlyAsp
180 185

559080" 25049560

SEQ ID NO: 4

SEQUENCE LENGTH: 1694

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: Genomic DNA

ORIGINAL SOURCE

ORGANISM: mouse

SEQUENCE DESCRIPTION:

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1 5
GAAGTGGGGTCTGGAGACTATGACTCCAACAAGGAACCCTGCTTCCGG 75
GluValGlySerGlyAspTyrAspSerAsnLysGluProCysPheArg
10 15 20 25
GATGAAAACGTCCATTTCAATAGGATCTTCCTGCCCACCATCTACTTC 123
AspGluAsnValHisPheAsnArgIlePheLeuProThrIleTyrPhe
30 35 40
ATCATCTTCTTGACTGGCATAGTCGGCAATGGATTGGTGATCCTGGTC 171
IleIlePheLeuThrGlyIleValGlyAsnGlyLeuValIleLeuVal
45 50 55
ATGGGTACCAGAAGAAGCTAAGGAGCATGACGGACAAGTACCGGCTG 219
MetGlyTyrGlnLysLysLeuArgSerMetThrAspLysTyrArgLeu
60 65 70
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HisLeuSerValAlaAspLeuLeuPheValIleThrLeuProPheTrp
75 80 85
GCAGTTGATGCCATGGCTGACTGGTACTTTGGGAAATTTTGTGTAAG 315
AlaValAspAlaMetAlaAspTrpTyrPheGlyLysPheLeuCysLys
90 95 100 105
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110 115 120
CTGGCCTTCATCAGCCTGGACCGGTACCTCGCCATTGTCCACGCCACC 411

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 125 130 135
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 140 145 150
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 155 160 165
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 205 210 215
 TGTTACTGCATCATCATCTCTAAGCTGTCACACTCCAAGGGCCACCAG 699
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 220 225 230
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 285 290 295
 AACCCCATCCTCTATGCCTTCCTCGGGGCCAAGTTCAAAGCTCTGCC 939
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559080" 250/9250

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330 335 340 345
TCCTCCAGTTTTTCACTCCAGCTAACCCTTATGCAAAGACTTATATAAT 1083
SerSerSerPheHisSerSer
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TGAAATGGTGGTAGAAATGCTGGGGTTTTTTTTTGTGTTGTTGTTTTTT 1611
CAGTTTTCAAGAGTAGATTGACTTCAGTCCCTACAAATGTACAGTCTT 1659
GTATTACATTGTTAATAAAAAGTCAATGATAAACTT 1694

SEQ ID NO: 5

SEQUENCE LENGTH: 20

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid (synthetic DNA)

FEATURE: 13, 15 (inosine)

SEQUENCE DESCRIPTION:

CTSMGTTTGK CMNTNKCYGA

SEQ ID NO: 6
SEQUENCE LENGTH: 26
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
FEATURE: 8, 9, 17 (inosine)
SEQUENCE DESCRIPTION:
TAGAKSANNG GRTTSANRCA RCGTG 26

SEQ ID NO: 7
SEQUENCE LENGTH: 25
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
SEQUENCE DESCRIPTION:
TCATCCCCCT GACTGATGTC CCCCT 25

SEQ ID NO: 8
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
SEQUENCE DESCRIPTION:
CCATCCTAAT ACGACTCACT ATAGGGC 27

SEQ ID NO: 9
SEQUENCE LENGTH: 30
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)

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SEQUENCE DESCRIPTION:

CGCGTCGACC ACAACATGCT GTCCACATCA 30

SEQ ID NO: 10

SEQUENCE LENGTH: 30

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid (synthetic DNA)

SEQUENCE DESCRIPTION:

CGCTCTAGAT TATAAACCAG CCGAGACTTC 30

SEQ ID NO: 11

SEQUENCE LENGTH: 29

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid (synthetic DNA)

SEQUENCE DESCRIPTION:

CGCGTCGACG TTACCATGGA GGGGATCAG 29

SEQ ID NO: 12

SEQUENCE LENGTH: 32

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid (synthetic DNA)

SEQUENCE DESCRIPTION:

CGCGCGGCCG CTTAGCTGGA GTGAAACTT GA 32

SEQ ID NO: 13

SEQUENCE LENGTH: 27

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

659030" 25049560

TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
SEQUENCE DESCRIPTION:
TAGCGGCCGC GTTGCCATGG AACCGAT 27

SEQ ID NO: 14
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
SEQUENCE DESCRIPTION:
GCGTCGACTA AGGGTTAGCT GGAGTGA 27

SEQ ID NO: 15
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
SEQUENCE DESCRIPTION:
CTGCACCTGT CAGTGGCTGA 20

SEQ ID NO: 16
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
SEQUENCE DESCRIPTION:
TAGATGAGGG GGATTGAGAC AACAGTG 27

SEQ ID NO: 17
SEQUENCE LENGTH: 359

006705029E60

TOPOLOGY: linear

ORIGINAL SOURCE

ORGANISM: mouse

SEQUENCE DESCRIPTION:

1 5 10 15

20 25 30

35 40 45

50 55 60

65 70 75 80

85 90 95

100 105 110

115 120 125

130 135 140

145 150 155 160

165 170 175

180 185 190

195 200 205

210 215 220

CysTyrCysIleIleIleSerLysLeuSerHisSerLysGlyHisGln
225 230 235 240
LysArgLysAlaLeuLysThrThrValIleLeuIleLeuAlaPhePhe
 245 250 255
AlaCysTrpLeuProTyrTyrValGlyIleSerIleAspSerPheIle
 260 265 270
LeuLeuGlyValIleLysGlnGlyCysAspPheGluSerIleValHis
 275 280 285
LysTrpIleSerIleThrGluAlaLeuAlaPhePheHisCysCysLeu
 290 295 300
AsnProIleLeuTyrAlaPheLeuGlyAlaLysPheLysSerSerAla
305 310 315 320
GlnHisAlaLeuAsnSerMetSerArgGlySerSerLeuLysIleLeu
 325 330 335
SerLysGlyLysArgGlyGlyHisSerSerValSerThrGluSerGlu
 340 345 350
SerSerSerPheHisSerSer
 355 359

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CLAIMS

1. A DNA encoding a polypeptide comprising an entire
sequence of the amino acid sequence as shown by
5 SEQ ID NO: 17 of Sequence Listing or a partial sequence
thereof, or a polypeptide comprising the polypeptide
described above, wherein any of the polypeptides has an
activity of a receptor capable of binding to a murine
PBSF/SDF-1.

10 2. A DNA encoding a polypeptide resulting from at least
one of deletion, addition, insertion, or substitution of
one or more amino acid residues in an entire sequence of
the amino acid sequence as shown by SEQ ID NO: 17 of
15 Sequence Listing or a partial sequence thereof, wherein
any of the polypeptides has an activity of a receptor
capable of binding to a murine PBSF/SDF-1.

20 3. A DNA comprising an entire sequence of the nucleotide
sequence as shown by SEQ ID NO: 1 of Sequence Listing or a
partial sequence thereof, or a DNA comprising the DNA
described above, wherein any of the DNAs encodes a
polypeptide having an activity of a receptor capable of
binding to a murine PBSF/SDF-1.

25

4. A DNA resulting from at least one of deletion, addition, insertion, or substitution of one or more bases in a DNA comprising an entire sequence of the nucleotide sequence as shown by SEQ ID NO: 1 of Sequence Listing or a partial sequence thereof, or a DNA comprising the DNA, wherein any of the DNAs encodes a polypeptide having an activity of a receptor capable of binding to a murine PBSF/SDF-1.

5. A DNA being capable of hybridizing under stringent conditions with the DNA of any one of claims 1 to 4, and encoding a polypeptide having an activity of a receptor capable of binding to a murine PBSF/SDF-1.

6. A polypeptide encoded by the DNA of any one of claims 1 to 5, wherein the polypeptide has an activity of a receptor capable of binding to a murine PBSF/SDF-1.

7. A polypeptide comprising an entire amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, or a polypeptide comprising the polypeptide described above, wherein any of the polypeptides has an activity of a receptor capable of binding to a murine PBSF/SDF-1.

8. A polypeptide resulting from at least one of deletion, addition, insertion, or substitution of one or more amino acid residues in an entire amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, wherein the polypeptide has an activity of a receptor capable of binding to a murine PBSF/SDF-1.

9. The polypeptide according to any one of claims 6 to 8, derived from a murine pre-B-cell line DW34.

10. An expression vector carrying the DNA according to any one of claims 1 to 5.

11. A transformant obtained by introducing the expression vector according to claim 10 into a host.

12. The transformant according to claim 11, wherein the host is a mammalian cell line.

13. A method for producing a polypeptide having an activity of a receptor capable of binding to a murine PBSF/SDF-1, characterized in that the method comprises culturing the transformant according to claim 11 or 12 under conditions capable of expressing the expression vector according to claim 10.

14. A monoclonal antibody against the polypeptide according to any one of claims 6 to 9.

15. A pharmaceutical composition for the use as an AIDS onset inhibitor or an HIV-1 infection inhibitor, comprising a murine PBSF/SDF-1.

16. Cells expressing the polypeptide according to any one of claims 6 to 9 and a human CD4 protein.

17. A method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, characterized in that the method comprises the steps of:

- (a) mixing the cells expressing the polypeptide according to any one of claims 6 to 9, or cells according to claim 16; a human T-cell-line-tropic HIV-1; and a substance to be screened, and incubating the resulting mixture; and
- (b) analyzing localization of an HIV-1 in the cells.

18. The method according to claim 17, wherein the step of analyzing localization of an HIV-1 is carried out by using a monoclonal antibody against a human T-cell-line-tropic HIV-1.

19. A method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, characterized in that the method comprises the steps of:

- 5 (a) mixing the cells expressing the polypeptide according to any one of claims 6 to 9, or cells according to claim 16; cells expressing an HIV-1 envelope protein; and a substance to be screened, and incubating the resulting mixture; and
- 10 (b) determining a level of the fusion of the above cells with the cells expressing an HIV-1 envelope protein.

20. A method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, or a PBSF/SDF-1 agonist or antagonist, characterized in that the method comprises the steps of:

- 15 (a) mixing the cells expressing the polypeptide according to any one of claims 6 to 9, or cells according to claim 16; a murine or human PBSF/SDF-1; and a substance to be screened, and incubating the resulting mixture; and
- 20 (b) determining an intracellular calcium ion level and/or determining a binding activity of an expressed polypeptide with the murine or human PBSF/SDF-1.

25 21. The method according to claim 20, wherein the

antagonist is a hematopoietic stem cell liberator.

22. A kit for detecting an AIDS onset or an HIV-1
infection, comprising the cells expressing the polypeptide
5 according to any one of claims 6 to 9, or cells according
to claim 16.

23. A method for detecting an AIDS onset or an HIV-1
infection, characterized in that the method comprises;

- 10 (a) mixing the cells expressing the polypeptide according
to any one of claims 6 to 9, or cells according to
claim 16 with sera, blood cells or blood of a patient
suspected to be infected with an HIV-1, and
incubating the resulting mixture, and
15 (b) analyzing localization of an HIV-1 in the cells or
determining a level of the fusion of the cells with
HIV-1-infected cells.

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ABSTRACT

The present invention pertains to a DNA encoding a polypeptide comprising an entire sequence of the amino acid sequence as shown by SEQ ID NO: 17 of Sequence Listing or a partial sequence thereof, or a polypeptide comprising the polypeptide described above, wherein any of the polypeptides has an activity of a receptor capable of binding to a murine PBSF/SDF-1; a polypeptide encoded by the DNA described above, wherein the polypeptide has an activity of a receptor capable of binding to a murine PBSF/SDF-1; cells expressing the polypeptide described above and a human CD4 protein; and a method of screening an AIDS onset inhibitor or an HIV-1 infection inhibitor, characterized by the use of the cells described above. According to the present invention, there can be provided a novel murine CXC chemokine receptor gene, a method of screening an HIV-1 infection inhibitor, and the like, each of which is useful in studies of a therapeutic agent for AIDS and the functional mechanism of HIV-1 infection.

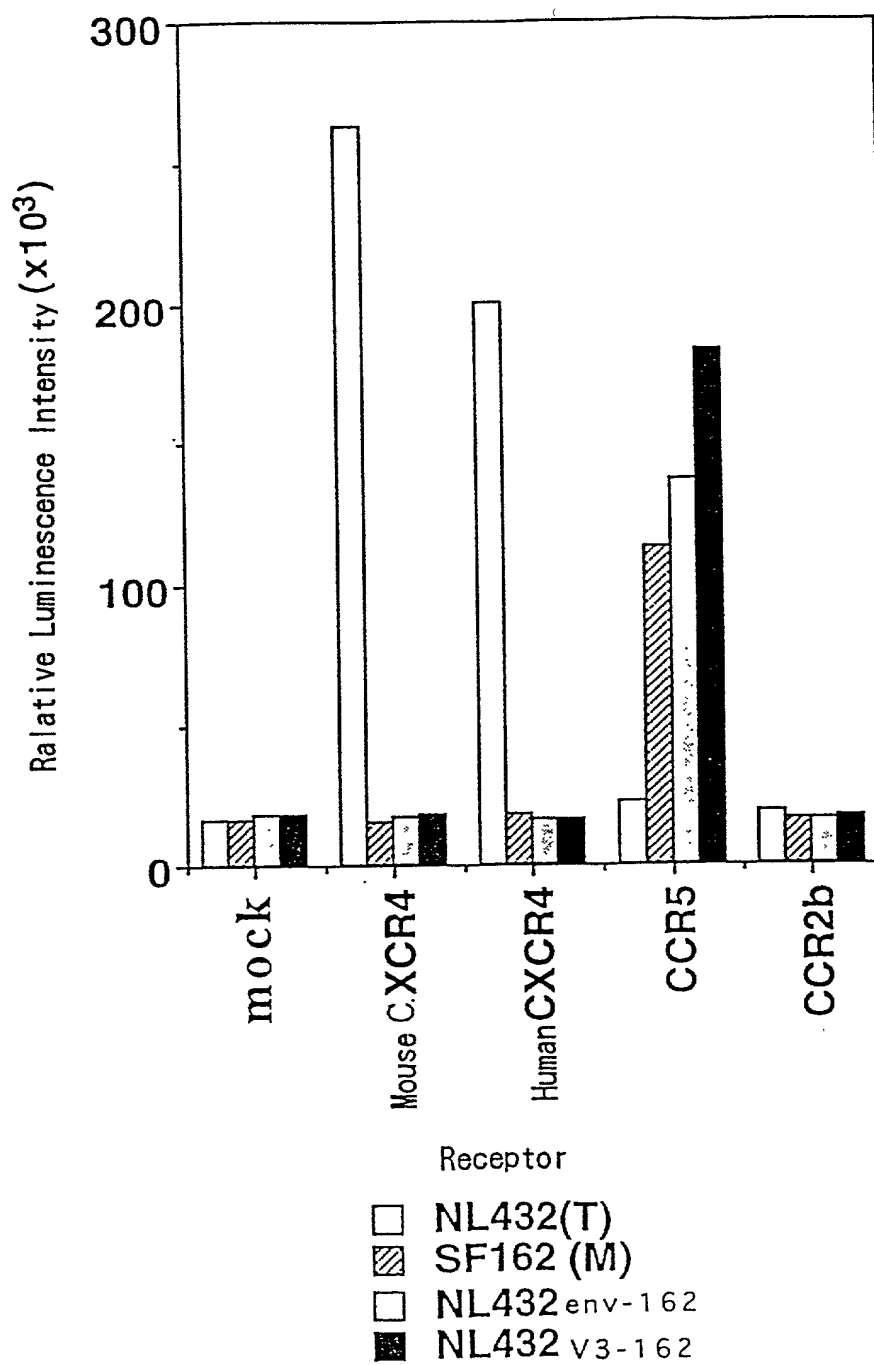


Fig. 13

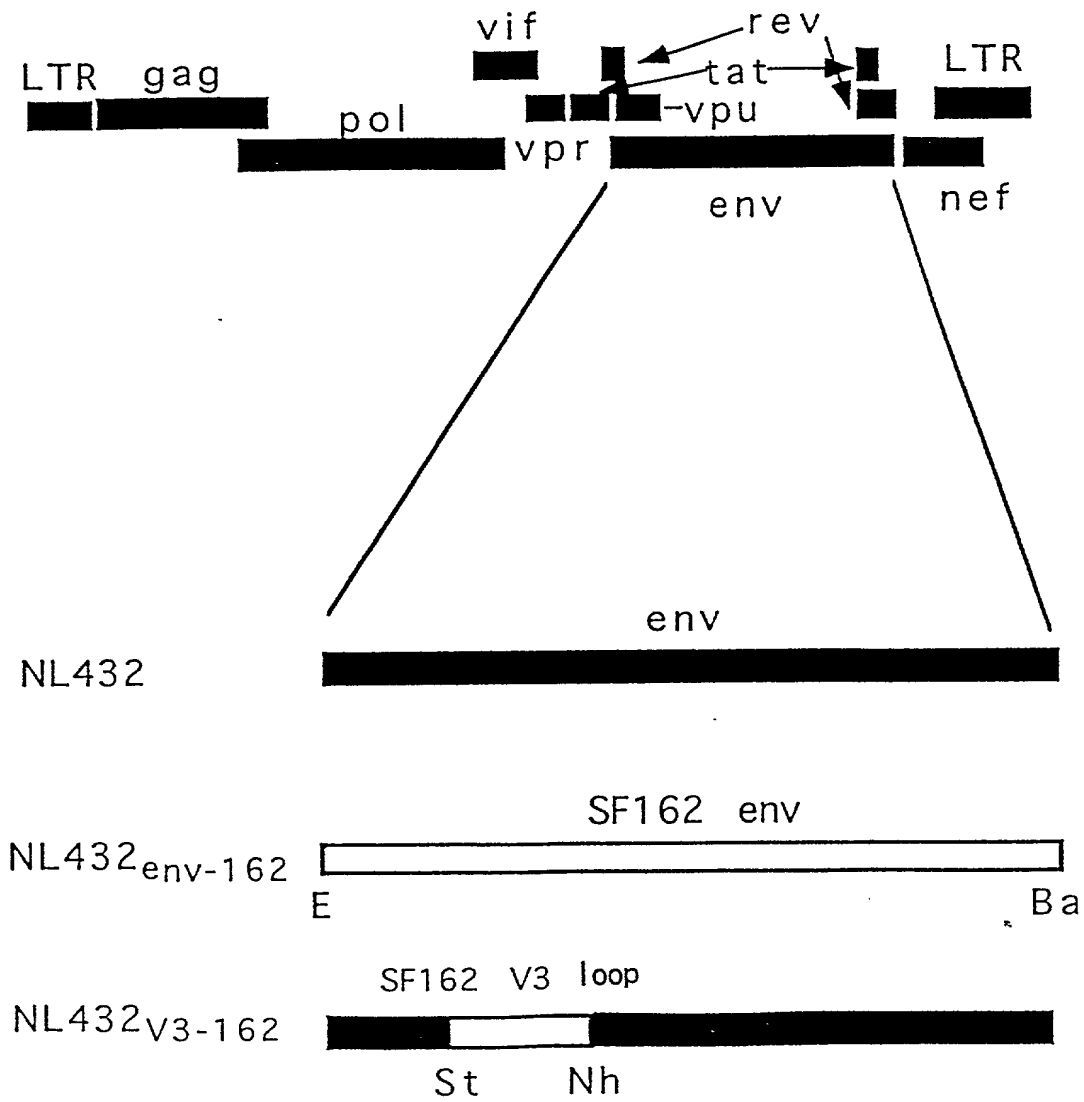


Fig. 12

C

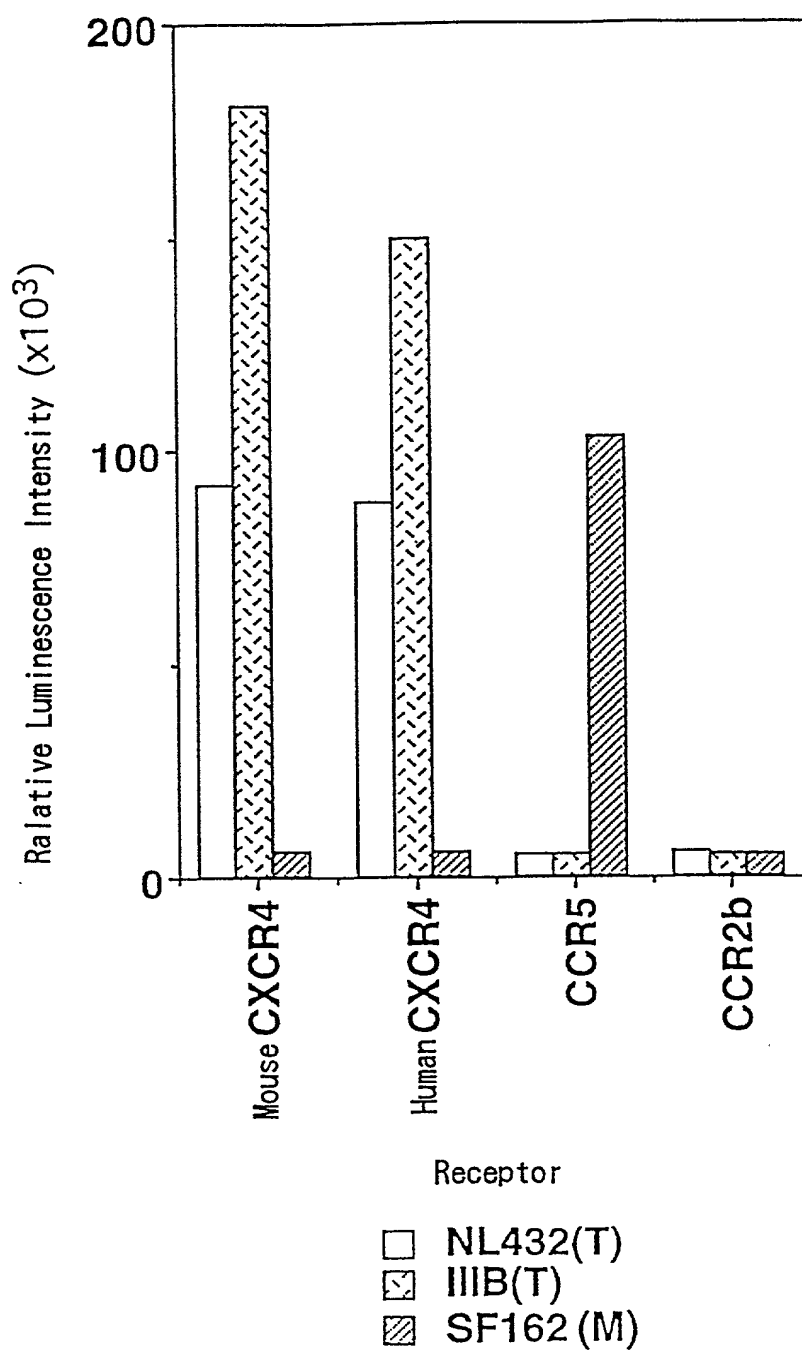


Fig. 11

B

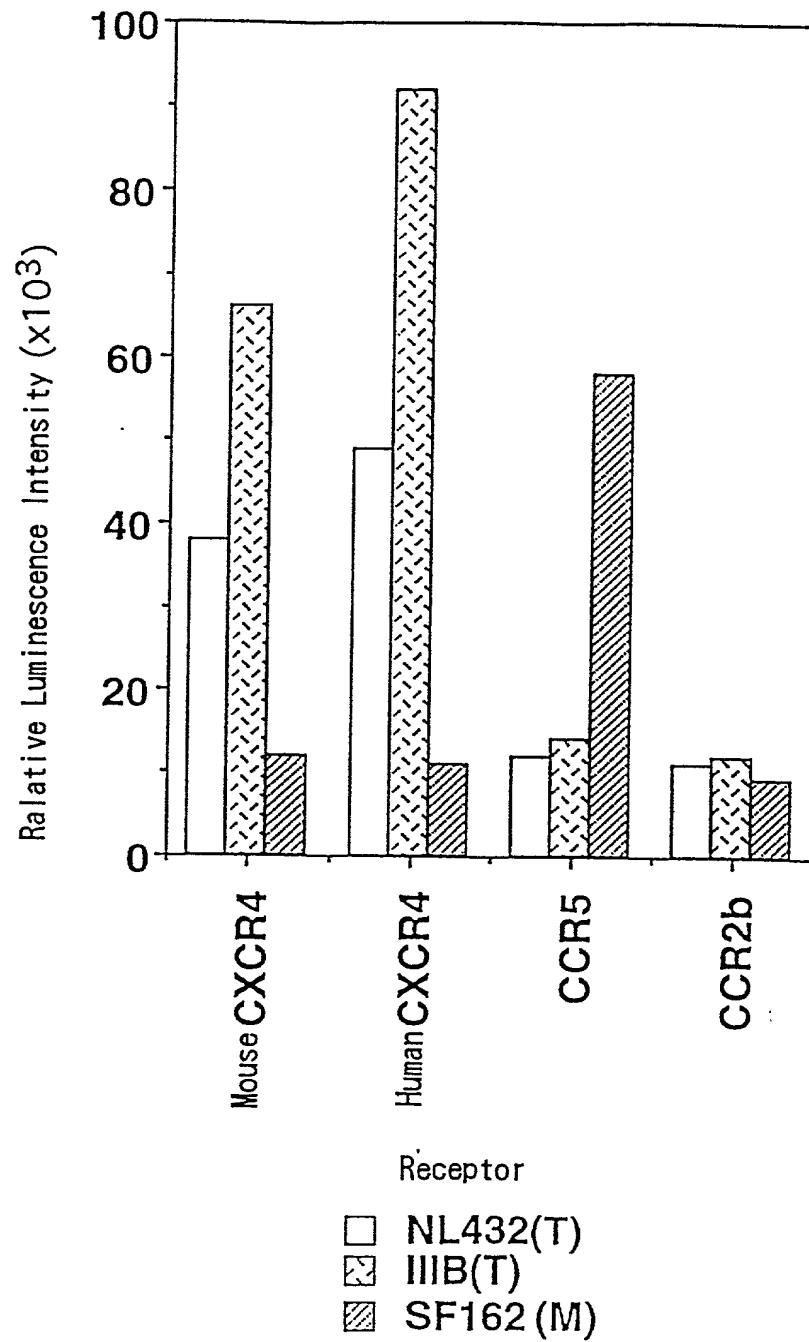


Fig. 10

A

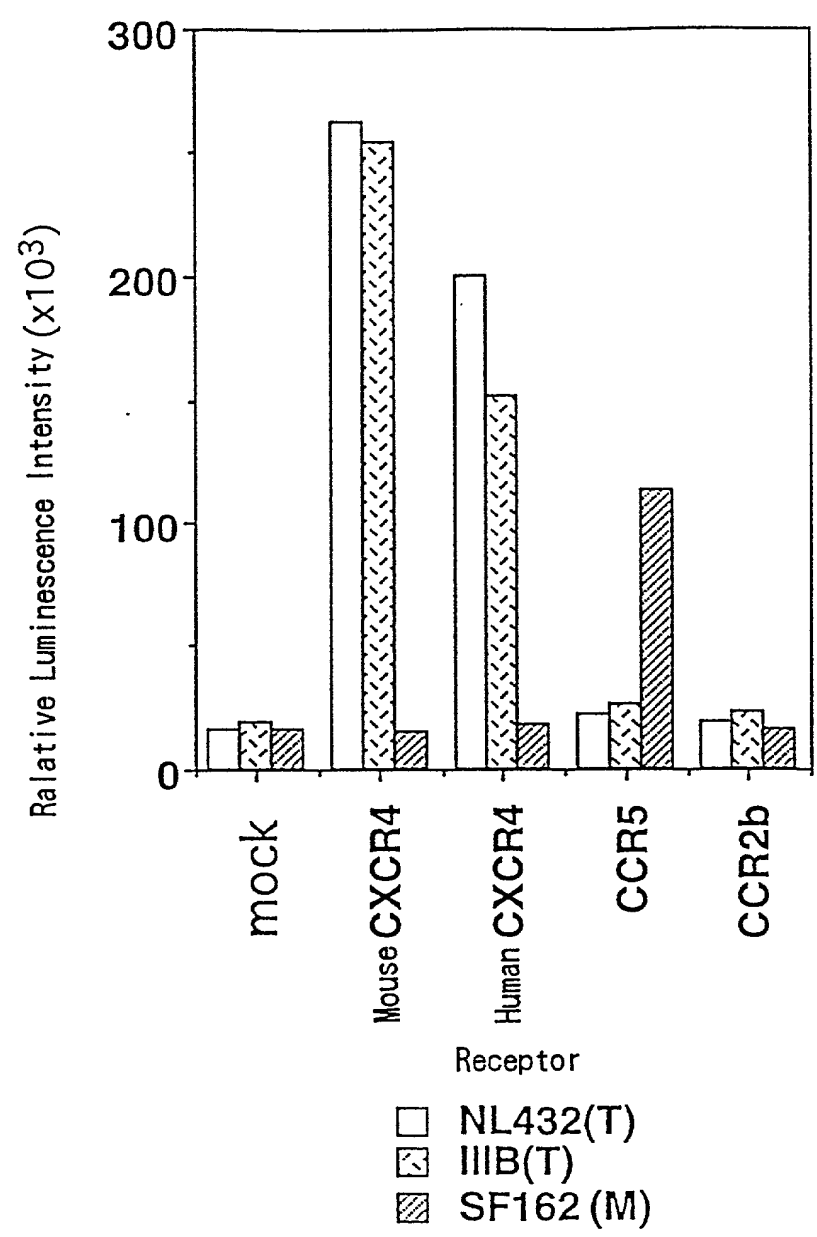


Fig. 9

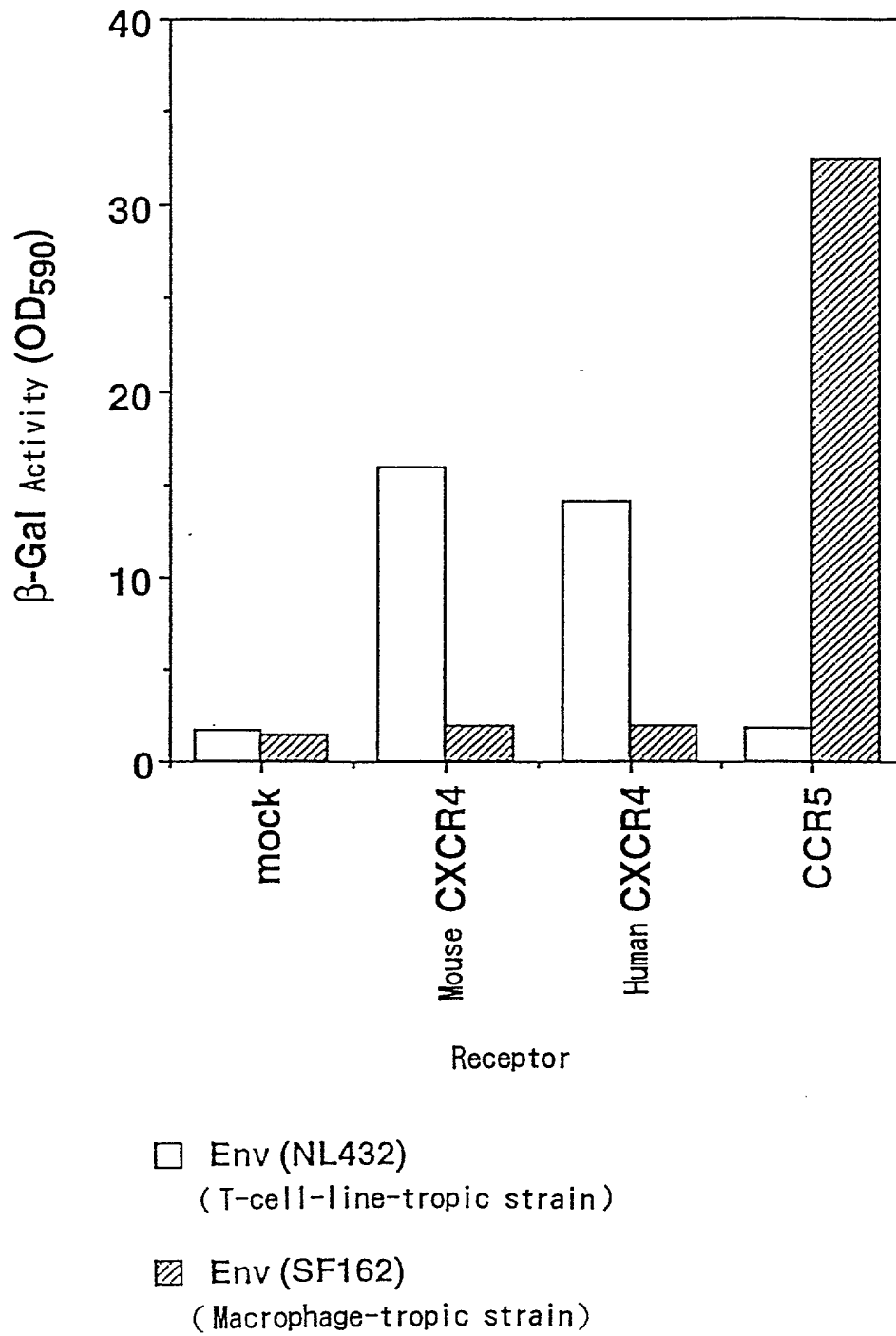


Fig. 8

CHO cells (mPBSF/SDF-1R+)

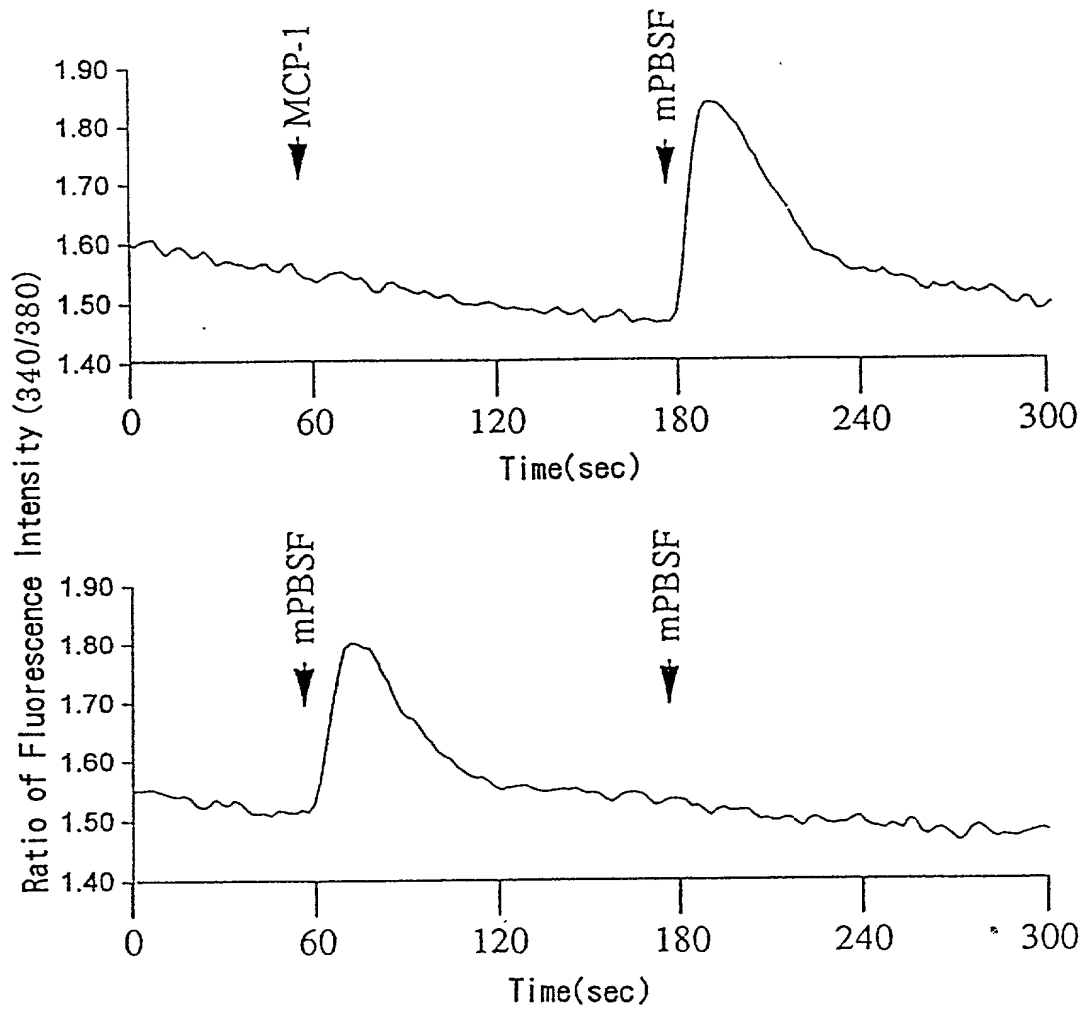


Fig. 7

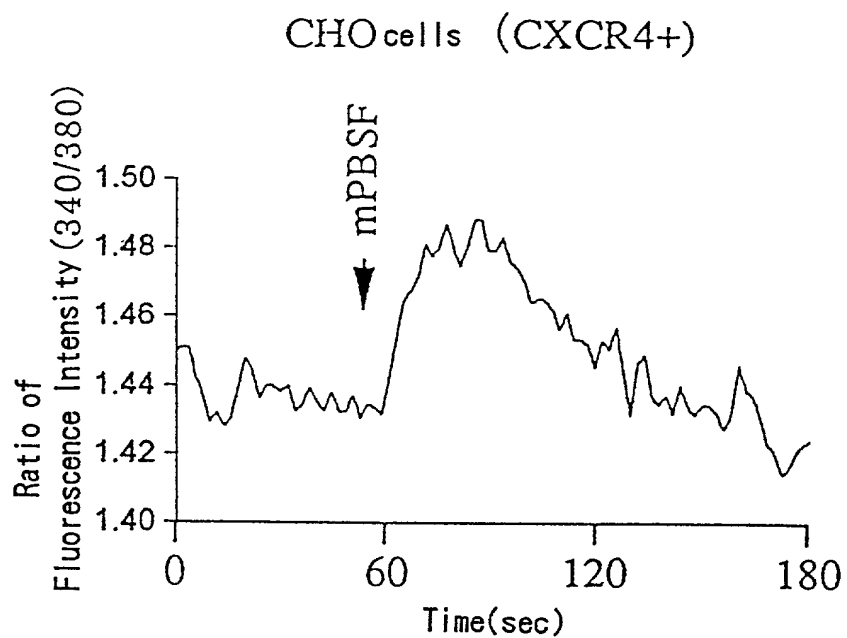


Fig. 6

659080 25049660

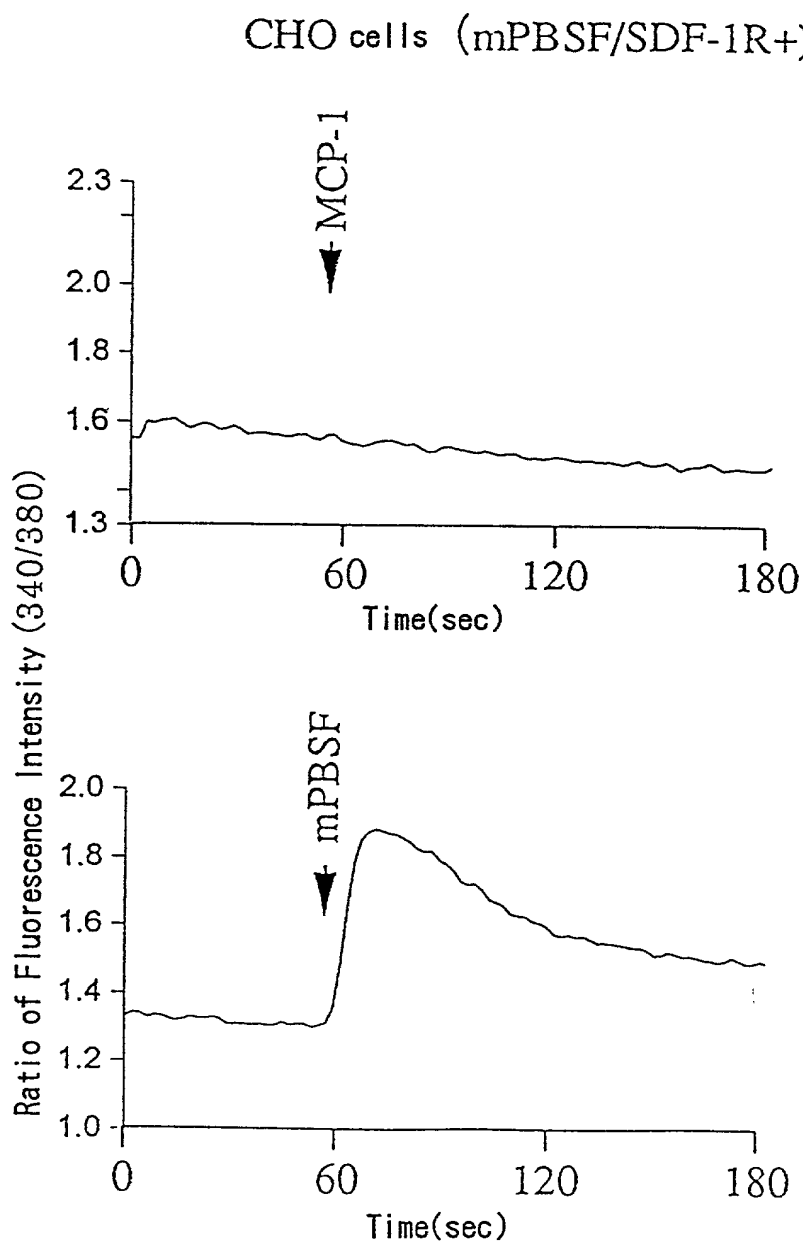


Fig. 5

CHO cells (CC CKR2B+)

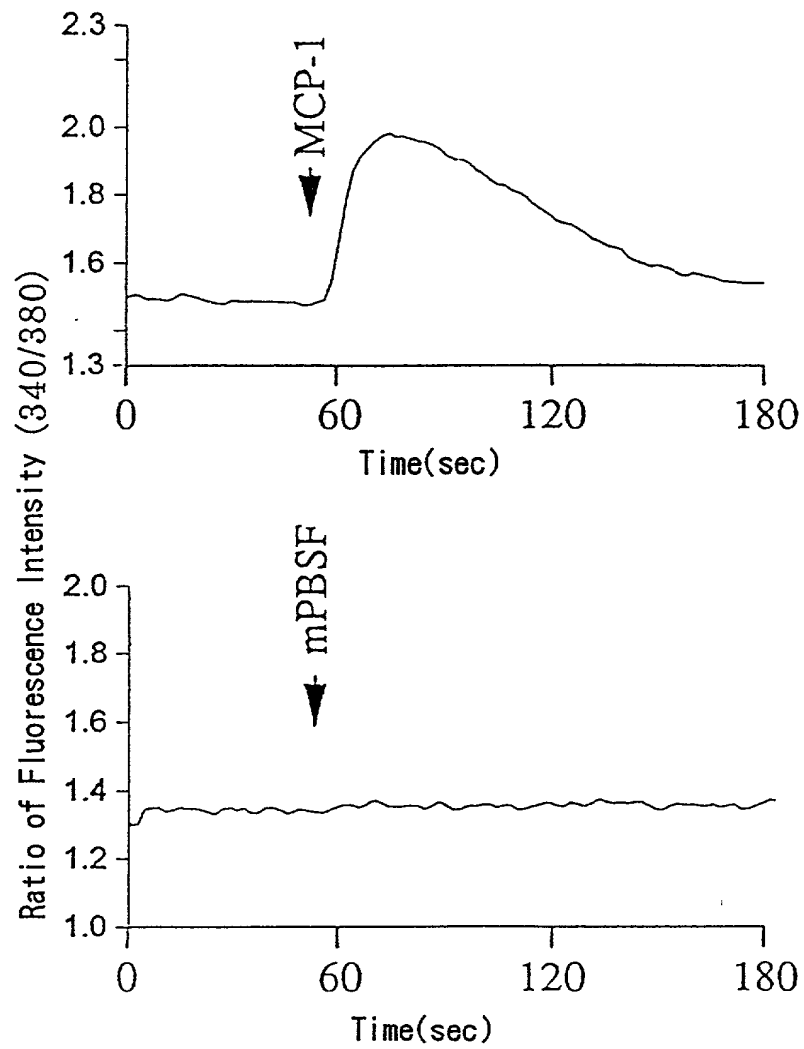


Fig. 4

CHO cells

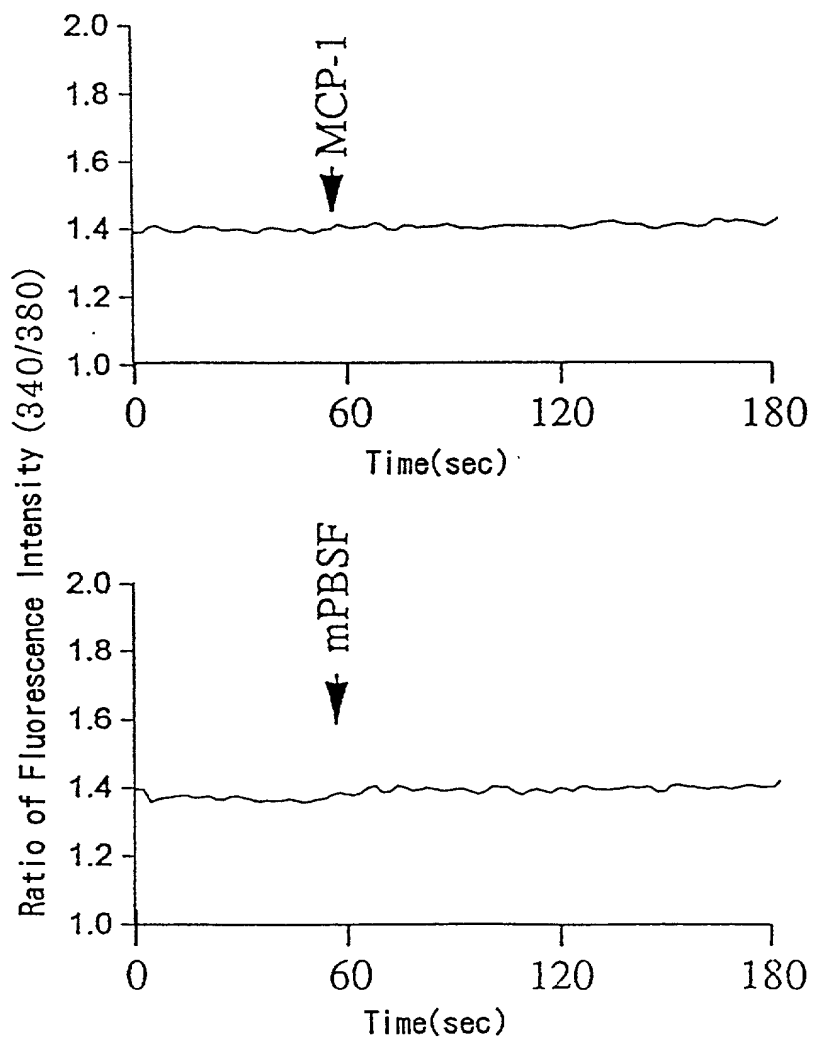


Fig. 3

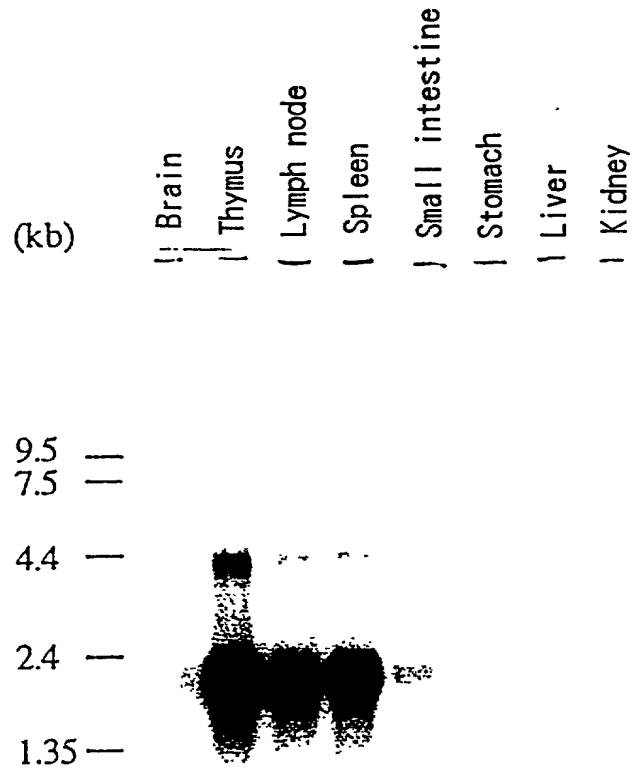
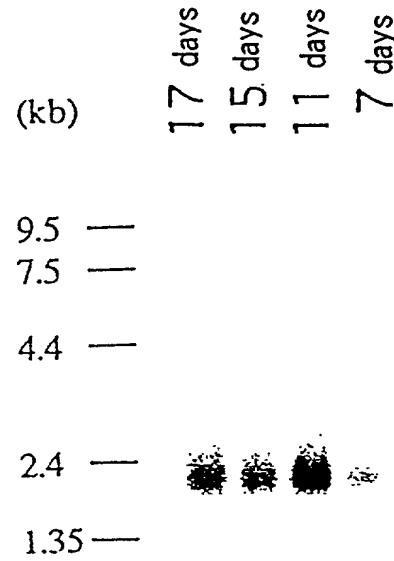
A**B**

Fig. 2

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Fig. 1 (Continued)

CTCGGTGTCTCTTGTGTCCAGCTCTGCAGCCTCGCGCGCGCCCTCCCGCCACGCCATGGACGCCAAGGTCGTCGCC
(79)
MetAspAlaLysValAla

GTGCTGGCCCTGGTGGCCGCTCTGTGCATCAGTGACGGTAAACCAGTCAGCCTGAGCTACCGATGCCCCCTGCCGGTTC
ValLeuAlaLeuValLeuAlaAlaLeuCysIleSerAspGlyLysProValSerLeuSerTyrArgCysProCysArgPhe

TTCGAGAGCCACATCGCCAGAGCCAAACGTCAAGCATCTGAAAAATCCTCAACACTCCAAACTGTGCCCTTCAGATTGTTGCA
(241)
PheGluSerHisIleAlaArgAlaAsnValLysHisLeuLysIleLeuAsnThrProAsnCysAlaLeuGlnIleValAla

CGGCTGAAGAAACAACAGACAAGTGTGCAATTGACCCGAAATTAAGTGGATCCAAGAGTACCTGGAGAAAGCTTTAAAC
ArgLeuLysAsnAsnAsnArgGlnValCysIleAspProLysLeuLysTrpIleGlnGluTyrLeuGluLysAlaLeuAsn

AAGTAAAGCACAAACAGCCCCAAAGGACTTTCCAGTAGACCCCCGAGGAAGGCTGACATCCGTGGGAGATGCCAAGGGCAGTGGT
(403)
Lys
GGGAGGAGGGCCCTGAACCCCTGGCCAGGATGGCCGGCGGACAGCAGTACTGGGGTCACTGCTAAGGTTTGCCAGCATAAA
GACACTCCGCCATAGCATATGGTACGATATTGCAGCTTATATTTCATCCCTGCCCTCGCCCGTGCACAAATGGAGCTTTTATA
ACTGGGGTTTTTCTAAGGAAATTGTATTACCCTAACCAAGTTAGCTTCACTCCCATTTCTCCTCATCTTCATTTTAA

Fig. 1

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International Application Number PCT/JP97/00299; and was
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Prior Foreign Application(s)

Priority Claimed

(Number)	(Country)	(Month / Day / Year Filed)	Yes	No
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>
_____	_____	_____	<input type="checkbox"/>	<input type="checkbox"/>

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_____	_____	_____
_____	_____	_____

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Full Name of Fourth
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